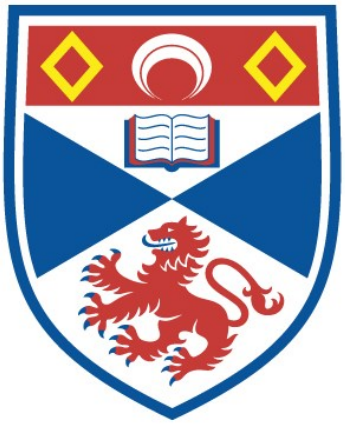
A STUDY OF SOME SOLUTION EQUILIBRIA OF BIOLOGICAL SIGNIFICANCE

Anna Mary Fiabane

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1976

A STUDY OF SOME SOLUTION EQUILIBRIA OF BIOLOGICAL SIGNIFICANCE

A THESIS

PRESENTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE FACULTY OF SCIENCE OF THE

UNIVERSITY OF ST ANDREWS

BY

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ABSTRACT

The stability constants of some lead(II)-ligand anion complexes already measured have been recalculated and others of some lead(II) and zinc(II) complexes have been measured by glass and chloride electrode potentiometry. These constants have been used in computer models of biological systems in order to assess the suitability of current and proposed therapeuticals as lead(II) sequestering agents *in vivo*. Glutathione is proposed as the most promising ligand for the treatment of plumbism.

Thermodynamic functions for some lead(II)-ligand anion complexes have been determined, by calorimetry and temperature variation of formation constants, and from these some complex structures in aqueous solution have been suggested.

The interaction of bovine serum albumin with lead(II) and copper(II) has been studied potentiometrically. The effect of some antirheumatoid arthritis drugs on the copper(II)-bovine serum albumin interaction has been observed by visible spectroscopy and molecular filtration. It is concluded that some of these drugs have the ability to release copper(II) from this protein bound situation.

A contribution has been made to an interlaboratory potentiometric study of the nickel(II)-glycinate complexing system and a comparison of results from several laboratories is presented.

DECLARATION

I DECLARE THAT THIS THESIS IS MY OWN COMPOSITION, THAT THE WORK OF WHICH IT IS A RECORD HAS BEEN CARRIED OUT BY MYSELF, AND THAT IT HAS NOT BEEN SUBMITTED IN ANY PREVIOUS APPLICATION FOR A HIGHER DEGREE.

THE THESIS DESCRIBES THE RESULTS OF RESEARCH PERFORMED IN THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF ST ANDREWS, UNDER THE SUPERVISION OF DR DAVID R WILLIAMS BETWEEN OCTOBER 1973 AND SEPTEMBER 1976.

ANNA M. FIABANE

CERTIFICATE

I HEREBY CERTIFY THAT ANNA M FIABANE HAS RESEARCHED UNDER MY SUPERVISION AND HAS FULFILLED THE CONDITIONS OF ORDINANCE GENERAL NUMBER 12 AND RESOLUTION OF THE UNIVERSITY COURT, 1967, NUMBER 1 AND IS QUALIFIED TO SUBMIT THIS THESIS IN APPLICATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

DAVID R WILLIAMS

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF ST ANDREWS

ACKNOWLEDGEMENTS

I WISH TO EXPRESS MY GRATITUDE TO DR DAVID R WILLIAMS FOR HIS ADVICE AND ENCOURAGEMENT THROUGHOUT THIS WORK.

THANKS ARE ALSO DUE TO ALL MY COLLEAGUES AND ESPECIALLY TO MR MURRAY L D TOUCHE FOR HIS WILLINGNESS TO HELP WITH COMPUTATIONAL PROBLEMS.

I THANK THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF ST ANDREWS FOR THE FACILITIES OFFERED AND THE SCIENCE RESEARCH COUNCIL FOR THE AWARD OF A RESEARCH STUDENTSHIP.

CONTENTS

LIST OF MAIN SYMBOLS

M	mol dm^{-3}
px	-log x
T	temperature (K)
R	the gas constant
F	the faraday
z	the number of electrons involved in a redox process
$^{T}E_{o}$	standard electrode potential
E_{o}	formal electrode potential
E	measured electrode potential
A	ligand
B	metal
H	proton
^{T}A	total concentration of A
^{T}B	total concentration of B
^{T}H	total concentration of H
a	concentration of free A
b	concentration of free B
h	concentration of free H
()	activity
f	activity coefficient
[]	concentration
I	concentration of ionic background salt
pqr	the complex $A_p B_q H_r$ where r < 0 indicates a hydroxyl group
$\bar{Z}$	the average number of ligands, A, bound to central group, B

$\bar{Z}_h$ the average number of protons, H, bound to ligand, A

w_k ionic product of water

β^o_{pqr} thermodynamic stability constant of the species $A_pB_qH_r$

β_{pqr} stoichiometric stability constant of the species $A_pB_qH_r$

K_p stepwise stability constant for the complexation reaction

$$A_{p-1}B + A \rightleftharpoons A_pB$$

Q_{corr} corrected heat change

$\Delta G^\ominus$ Gibbs free energy change*

$\Delta H^\ominus$ enthalpy change*

$\Delta S^\ominus$ entropy change*

Graphs are plotted in the order

△ ▽ ⊡ ◇ ⊙ ⊞ ⊠ ⊕ ⊗ ✠

* with 3.00M $NaClO_4$ as the standard solvent

CHAPTER 1

INTRODUCTION

CONTENTS

CHAPTER 1

INTRODUCTION

General

In 1946 the World Health Organisation defined "health" as "a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity". At least twenty-five elements [1] are necessary for man's achievement of this state of health.

The elements of organic matter, H, C, N, O, P and S, constitute by far the greatest proportion of the body's atoms. The remaining biological elements may be classified as main group, Na, K, Mg, Ca and Cl, which, as ions, are usually mobile, or trace elements, Zn, Fe, Cu, Mo, Co, Mn, Cr, V, Si, Sn, Se, F, Br and I, which usually have fixed chemical neighbours in a biological system.

People living in a modern industrial society may be exposed to excessive concentrations of both essential and non-essential metals as well as to non-metal pollutants such as sulphur dioxide and ligands such as nitrilotriacetate. The metallic pollutants causing most concern at the moment are lead, cadmium and mercury.

Lead Poisoning and its Treatment

Lead is, and always has been, naturally present in man's environment and so in man himself. However, as with other metallic contaminants, man is redistributing it from fairly remote deposits to populated areas where it may accumulate to hazardous levels.

World production of primary ore lead in 1970 [2] was 3.8×10^{12}g and it is estimated that 2.4×10^{11}g of lead enters the oceans each year [3]. In spite of this extremely high turnover the last recorded industrial death attributed to lead was in 1956 [4].

The average adult daily intake of lead from food [5] is 220ug but only 10% of this is absorbed. The fraction of inhaled lead which is absorbed depends on solubility and particle size and has been estimated as 10-50% [6]. The major source of this atmospheric lead appears to be the combustion of leaded petrol, [4,6,7] the products of which are complex halides which are converted in the atmosphere to oxides and carbonates [8].

Lead is present in the human body only as an environmental contaminant and appears to perform no normal biological function [9]. A stable isotope study [10] in the normal human has not been able to discover a homeostatic mechanism for the maintenance of the lead concentration in blood.

The natural mean lead content of blood has been estimated [11] as 2.5ng cm^{-3}. However, the mean blood lead level of 'normal' children in Glasgow has been measured [12] as 300ng cm^{-3} and of a control group of Manchester children [13] as 310ng cm^{-3}. In the U.K. the lower mean blood lead level in children showing lead poisoning

is [14] 400ng cm^{-3} but the suggested 'danger level' is [15] 250ng cm^{-3}. Thus lead levels in the general population appear to be much closer to the levels for overt clinical poisoning than for any other chemical pollutant.

About 95% of the body burden of lead is stored in bone [16] as the relatively insoluble lead triphosphate [6]. This is a non-diffusable form of the metal which is non-toxic. It is the small fraction of lead which is mobile in the soft tissues which is responsible for the acute toxic effects [17].

Inorganic lead is a general metabolic poison which is cumulative in man [7]. It is believed that lead(II) displaces essential transition metal ions from biologically important groups (predominantly sulphydryl groups). The inhibition of the enzyme system necessary for haem formation is thought to operate by the blocking of sulphydryl groups but there is no direct evidence for this [6]. Of the many effects of lead poisoning on intermediary metabolism the production of anaemia is the most common and may be used diagnostically.

In children neural tissue is particularly sensitive to lead and about 25% of the children who survive an attack of acute encephalopathy sustain severe permanent brain damage. An even more worrying factor is that the blood lead level at which central nervous system injury in children can occur is still debated and correlations between impaired mental functioning and levels well below the clinical threshold have been reported [18].

The accepted treatment for plumbism is with calcium disodium ethylenediaminetetraacetate ($CaNa_2$edta) and 3,3-dimethylcysteine

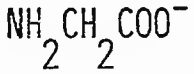
(D-penicillamine) (for formulae see figure 1). 2,3-Dimercapto-propanol(BAL) is not generally used because of its toxic side effects [3,19]. Treatment with $CaNa_2$edta, however, causes marked depletion of essential zinc in the body [20,21]. Computer models have been used [22] to show that D-penicillamine also is insufficiently selective and may remove zinc from the body.

Part of the research reported in this thesis was, thus, to suggest a ligand of improved selectivity for the removal of lead from the body.

Ligands Studied

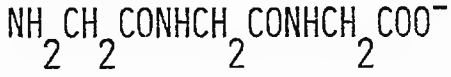
The formulae of the ligands used in this study are shown in figure 1.

Originally the complexing of eight representative amino acid anions to lead(II) had been studied [22] i.e. asparaginate, aspartate, cysteinate, glutaminate, histidinate, phenylalaninate, serinate and tryptophanate. The formation constants for these systems have been recalculated in the light of new computational techniques and some additional measurements have been carried out on the lead-aspartate and lead-cysteinate systems. The ligands normally used for treating plumbism, edta and D-penicillamine, have also been studied under the same experimental conditions, as has glutamate. For purposes of comparison it was also necessary to measure the zinc(II) complex formation constants for some of these ligands.

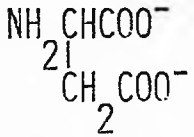


GLYCINATE(gly)

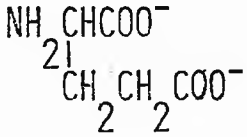
GLYCYLGLYCINATE(glygly)



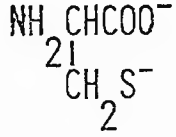
GLYCYLGLYCYLGLYCINATE(glyglygly)



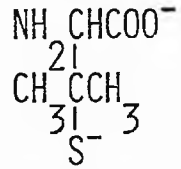
L-ASPARTATE(asp)



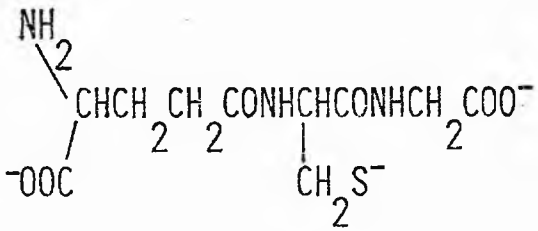
L-GLUTAMATE(glu)



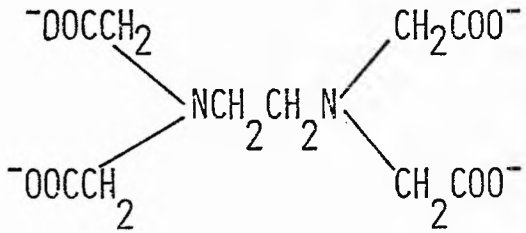
L-CYSTEINATE(cys)



D-PENICILLAMINATE(D-pen)



GLUTATHIONATE(gsh)



ETHYLENEDIAMINETETRAACETATE(edta)

FIGURE 1 : LIGANDS USED IN THE POTENTIOMETRIC OR CALORIMETRIC STUDY

Determination of Formation Constants

J. Bjerrum [23] showed that stepwise equilibria are generally set up when a ligand is added to a solution containing a metal aquo ion. Most ligands are weak acids and so there is a competition between the metal ion and the proton for the ligand anion. Thus, the complexing equilibria are dependent upon the hydrogen ion concentration and can be followed by monitoring the emf of an electrode reversible to hydrogen ions such as the glass electrode.

The emf method for measuring concentrations of metal ions or protons was first used at the end of the nineteenth century [24,25] and today is the most common technique applied to equilibrium systems. This method, of course, allows calculation of stability constants only if the complexes dissociate at pH values for which the proton concentration is accurately measurable. The emf titration method provides both high precision and a large number of experimental data points, both of these conditions being particularly required if other than simple mononuclear species are present.

The sensitivity of the glass electrode to the surrounding medium was first noted in 1906 [26] but not quantified till 1909 [27]. A detailed discussion of these electrodes is given by Bates [28].

The glass electrode, which is now an everyday piece of laboratory equipment, is unusual in not being influenced by oxidising and reducing agents and not being poisoned by heavy metals. Apart from the imperfect hydrogen ion response at the end of the pH scale, the most serious disadvantages of this electrode are its high electrical resistance and its low durability especially in alkaline solution.

Computer Models

Having obtained formation constants for all the complexing reactions of interest, it is then possible to simulate the *in vivo* complexing competition between essential metal ions and ligands and to indicate the influence of a polluting metal ion and the effect of any ligands suggested as therapeuticals for its removal. Some very powerful computer programs are now available to calculate these equilibrium concentrations of species. For example, one [29] can calculate the concentrations of up to 5,000 species present in a blood plasma model.

These computer models are, of course, far removed from the true *in vivo* situation and can only be used to make suggestions as to which ligands it may be worthwhile putting forward for animal trials.

Our initial model studies [22] indicated that a more selective drug for the treatment of lead poisoning might be a peptide containing cysteinyl,histidyl and aspartyl residues. The nearest peptide to this which is commercially available at reasonable cost is L-γ-glutamyl-L-cysteinylglycine(glutathione). Thus the lead(II) and zinc(II) complex formation constants of glutathionate were measured and their inclusion in further models [30] showed that we would, indeed, expect glutathione to be more selective than edta for the removal of excess body lead.

Having focussed attention on glutathionate, it was thought desirable to obtain further information about its bonding to lead(II) in solution.

Determination of Thermodynamic Functions

All processes involving bond breaking or making, be they chemical or biological, are accompanied by heat changes. Measurements of these heat changes yield values for the enthalpies of reaction and so can be used in conjunction with potentiometric measurements to calculate the entropies of reaction. Values of $\Delta H^{\ominus}$ can give information concerning bond energies in solution and $\Delta S^{\ominus}$ is a measure of the interaction with the solvent [31].

The preferred method for the determination of $\Delta H^{\ominus}$ values is direct measurement by incremental titration calorimetry. Calorimeters were first used more than a hundred years ago [32] and many different designs of solution calorimeter have been described [33]. However, a titration procedure was first used only in 1955 [34] and the first description of the incremental thermometric titration technique was in 1959 [35].

The great advantage of the titration approach is that a large amount of data can be collected in a reasonable time and then one can adopt a least-squares approach to data analysis. It is believed [31] that an incremental titration method is more accurate than the continuous titration technique because it allows the contents of the calorimeter to reach equilibrium for each point and also allows the contents to be returned to the initial temperature between points.

The thermodynamic functions have been determined for the interactions of protons and lead(II) with glutathionate and, for purposes of comparison, with glycinate, glycylglycinate, glycylglycylglycinate and cysteinate. Unfortunately, for the lead(II)-ligand

interactions direct calorimetry could not be used because of the interference of insoluble complexes and lead-hydroxy species [36,37]. Thus the van't Hoff method was resorted to i.e. enthalpies were calculated from the temperature variation of the formation constants. This method is much less satisfactory because of its inherent, and often unjustified, assumption that $\Delta H^{\ominus}$ does not vary within the temperature range studied and also because formation constants do not change greatly over this temperature range and so have to be measured with great accuracy to give reliable values for $\Delta H^{\ominus}$.

These thermodynamic results have then been used to suggest structures for the various complexes present in the solution.

Since blood plasma is about 0.1009 M in chloride ions, [38] it was considered pertinent to determine formation constants for the interaction of chloride ions with lead(II) under our experimental conditions. This was done by use of a silver/silver chloride electrode, which responds to the activity of chloride ions in solution [39], as well as a glass electrode.

Blood also has a high protein content but it has been shown [29] that this should not effect the distribution of metals among the small ligand complexes. However, the inclusion of human serum albumin in the copper-zinc-amino acid model system [40] has the effect of almost halving the concentrations of the zinc-small ligand complexes.

We have chosen bovine serum albumin as a representative of the blood proteins and the order of its binding to lead(II), zinc(II) and copper(II) has been determined potentiometrically.

Only 2% of plasma copper is bound to serum albumin the remainder being bound strongly, in a non-exchangeable form, to caeruloplasmin [41,42]. However, this small amount of copper(II)-human serum albumin complex is the exchangeable form of copper *in vivo* and is in rapid equilibrium with copper in the tissues [43]. It is known [44,45] that in blood plasma there is a negligible amount of free copper(II) and it is considered [46,47] that there is an equilibrium between serum albumin and amino acid bound copper(II) which may be mediated through ternary complexes [45]. It has also been shown [48] that low molecular weight copper(II) complexes may transport copper between the blood and the tissues.

Copper and Arthritis

A number of folk remedies for arthritis (a disease characterised by pain and joint inflammation) involve substances of high copper content and, in fact, there is now a great deal of evidence connecting copper with rheumatoid arthritis and other inflammatory diseases.

Rheumatoid arthritis patients show a high level of copper in their serum and an increased urinary excretion of this metal [49]. However, there is a suggestion [50] that in this disease there is an insufficient supply of copper in the right form for the normal copper-dependent metabolic processes of tissue maintenance.

Most of the drugs used in the treatment of rheumatoid arthritis and other collagen diseases are capable of binding metals [9] and it

has been shown [50] that the copper chelates of known anti-arthritic drugs may be up to twenty times more active than the drugs alone. Copper chelates of non-active drugs also may be more active than copper alone.

Several mechanisms for the involvement of copper have been postulated, for example

(i) [51] anti-pyretic agents such as salicylate may transport copper in a complexed form back to its intracellular site.

(ii) [52] anti-arthritic drugs, possibly via their copper complexes, may inactivate the enzyme PGE_2 synthetase and so divert production to the less inflammatory PGF_2.

(iii) [50] repair at sites of inflammation requires extracellular cross-linking of collagen and elastin and the enzyme responsible for this, lysyl oxidase, is copper-dependent.

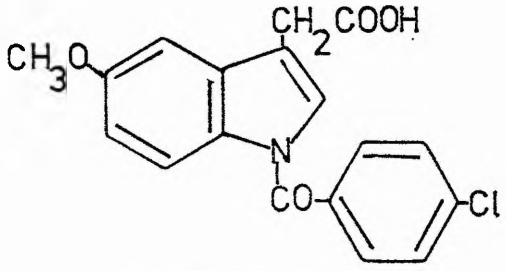
It is also known [41], however, that human serum albumin will bind to a large variety of drugs, for example, 75% of salicylic acid is bound to plasma protein [53].

For the drugs used to treat arthritis the following order of potency (for adjuvant arthritis in rats) has been given [54].

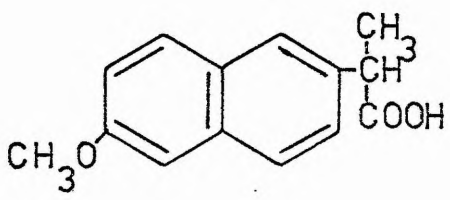
Indomethacin > Naproxen ≥ Ketoprofen > Fenoprofen ≥ Phenylbutazone > Aspirin.

(The formulae of these compounds are shown in figure 2.)

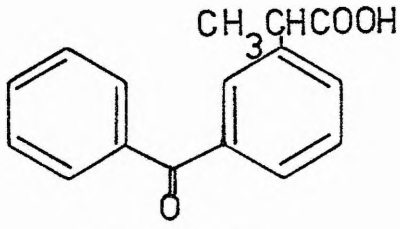
About 99% of each of these drugs is bound to human serum albumin [54] and so it is possible that they may affect the binding of copper to human serum albumin in such a way as to release it to take part in any of the mechanisms (i) - (iii) above.



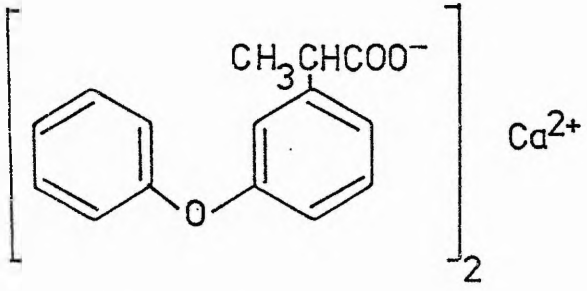
INDOMETHACIN



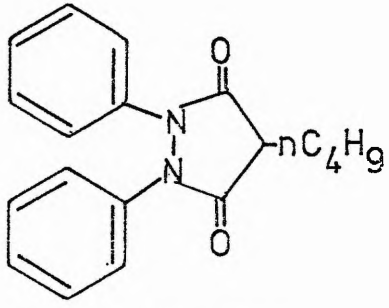
NAPROXEN



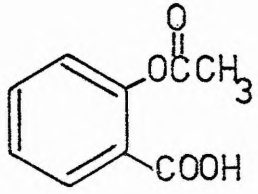
KETOPROFEN



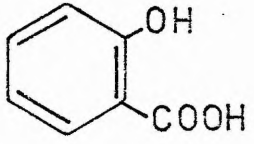
FENOPROFEN



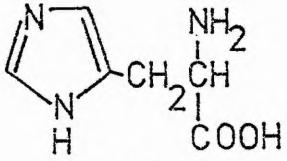
PHENYLBUTAZONE



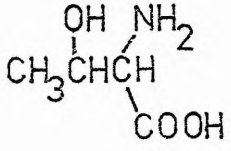
ASPIRIN



SALICYLIC ACID



HISTIDINE



THREONINE

FIGURE 2 : LIGANDS USED IN THE MOLECULAR FILTRATION STUDY

Copper-Bovine Serum Albumin-Drug Interactions

The anti-arthritic drugs mentioned above were studied, as was salicylic acid, since it is believed that this is the active form of aspirin *in vivo*, and histidine and threonine for purposes of comparison. Unfortunately, these drugs are not sufficiently soluble to allow the use of the potentiometric technique.

An initial trial using visible spectrophotometry in 90% ethanolic solution showed that the copper binding might be affected by some of the drugs. However, under these conditions the conformation of the protein and hence its complexing behaviour may be completely different from that in aqueous solution. In order to carry out a study *in aquo* we require

(i) a method for separating protein bound and small molecule bound copper fractions

and

(ii) a method for analysing very low concentrations of copper.

The separation technique chosen was molecular filtration. This is simply a method for separating dissolved molecules on the basis of size by passing a solution through a fine filter. The nominal molecular weight limit was chosen such that the drug-copper complexes would pass through but the bovine serum albumin-copper complexes would be retained.

The filtrate was then analysed for copper by atomic absorption spectrophotometry and so the fraction of the copper which was bound to the protein could be calculated.

The first publication concerning atomic absorption spectrophotometry was in 1957 [55] and commercial equipment, capable of routine operation, was introduced in 1963. The element which is being analysed is dissociated from its chemical bonds, by burning the sample in a flame, to give an unexcited, unionised ground state which can then absorb radiation at discrete lines of narrow band width. This absorption of radiation is measured and so the concentration of the element determined.

"Challenge" Project

Potentiometric studies of the same system in different laboratories unfortunately arrive at differing results more often than could be wished. Thus an interlaboratory study has been set up on the 'simple' nickel(II)-glycinate system in order to perhaps improve general techniques or highlight specific deficiencies.

In February 1975 the Organising Committee of the 'Thermodynamics of Complexes Group' from Italian Universities met in Rome to discuss the possibility of studying a single system in different laboratories in order to compare results obtained using basically the same experimental methods but with different techniques for preparing and analysing solutions and computing results. The Group met again in Padova in June 1975 in order to decide on the experimental conditions which should be used.

The conditions chosen involved the use of 1.00 M sodium chloride as the ionic background salt. Since our normal background salt is sodium perchlorate the experiments were repeated in this medium in order to determine how the change in background ions affects the formation constants obtained.

"Challenge" project results from several laboratories are now available and will be discussed in Chapter 9.

CHAPTER 2

THEORETICAL CONSIDERATIONS

CONTENTS

CHAPTER 2

THEORETICAL CONSIDERATIONS

Our aim in the study of an equilibrium between protons, ligands and metals is to completely define the system in terms of formation constants for all the species present. These formation constants may then be used to calculate the equilibrium concentration of each species. The computational methods used to achieve these aims will be discussed in the following chapter.

Formation Constants

Consider the formation of the species A_pB_q. In aqueous solution the species A, B and A_pB_q will be associated with a number of water molecules and so the overall equilibrium occurring can be represented as

$$pA(H_2O)_w + qB(H_2O)_x \rightleftharpoons A_pB_q(H_2O)_z + (pw + qx - z)H_2O \qquad (1)$$

(for simplicity, all charges are omitted).

In practice the activity of free water can be assumed constant and so the water of hydration is omitted from the equations to give

$$pA + qB \rightleftharpoons A_pB_q \qquad (2)$$

The activity of the species A_pB_q, formed in the solution at a given temperature, can be related to the activities of A and B by the law of mass action [56].

$$\beta^{o}_{pq} = \frac{(A_pB_q)}{(A)^p(B)^q} = \frac{[A_pB_q]}{[A]^p\,[B]^q} \times \frac{f_{A_pB_q}}{f_A^p\, f_B^q} \qquad (3)$$

where β^{o}_{pq} is the 'thermodynamic stability constant' for the species A_pB_q and f are activity coefficients. Thus, in order to determine β^{o}_{pq}, we must know the activities of all the species present. These may be calculated or, since activities and concentrations become equal at zero ionic strength, a series of measurements at low ionic strength may be extrapolated to infinite dilution. Both of these processes require a great deal of work and neither is satisfactory except for very simple systems.

However, if activity coefficients can be held constant, to within the limits of experimental error, then the 'stoichiometric stability constant' can be defined as

$$\beta_{pq} = \frac{[A_pB_q]}{[A]^p[B]^q} \qquad (4)$$

Stoichiometric stability constants are used in this work but this restricts comparisons to constants determined in the same medium.

The Ionic Medium Method

The ionic medium method is basically that of Bjerrum [23] and Leden [57] who suggested that activity coefficients could be held constant

if the concentrations of reactants and products were small compared to those of an inert electrolyte. This method has since been thoroughly investigated by Biedermann, Sillén and Ginstrup [58,59,60].

The requirements for the inert electrolyte are

(i) that it does not interfere with the reaction under investigation

(ii) it is sufficiently soluble to give the desired high background concentrations

(iii) it is available in a state of very high purity. Since the background salt is present in a great excess over the reacting species a small amount of impurity may be significant.

In order to avoid ion-pairing effects we choose perchlorate as the anion since the strengths of ion-pair bonds are in the order [32]

$$SO_4^{2-} > Cl^- > Br^- > I^- > NO_3^- > ClO_4^-$$

The ionic mobilities of the potassium and perchlorate ions are similar and thus our first choice of salt would be potassium perchlorate. However, this salt is insoluble and so sodium perchlorate is, in fact, used.

Having decided on an inert electrolyte we now need to choose the concentration to be used. In this laboratory a comparison [61] has been made between the two sets of conditions 25°C, 3.00M$NaClO_4$ and 37°C, 0.150M$NaClO_4$.

The disadvantages of 25°C, 3.00M$NaClO_4$ are

(i) these are far removed from biological blood plasma conditions which approximate to 37°C, 0.150MCl^-.

(ii) any final trace of impurity left in the Na ClO_4 is emphasised when the background salt is 3.00M.

(iii) even though the ClO_4^- ion has little tendency to ion-pair, at this high concentration some ion-pairing may occur [62].

(iv) the lower the temperature, the higher the amino group protonation constants and at 25°C they may be taken outside the working range of the glass electrode.

The disadvantages of 37°C, 0.150M$NaClO_4$ are

(i) a considerable amount of volumetric glassware needs to be recalibrated.

(ii) unless the complete system of vessel and electrodes is thermostatted at 37°C, condensation occurs in the cooler parts of the system and the electrothermal effect in the electrodes can cause an error of up to 3mV. [63]

(iii) the tubing linking the burette to the titration vessel should also be maintained at 37°C to minimise temperature fluctuations in the vessel.

(iv) a background salt concentration of 0.150M permits a change of only 0.008M in ion concentration without a significant variation in the activity coefficients [59].

(v) ion-responsive electrodes, such as amalgams, are less stable at higher temperatures.

(vi) the higher temperature may accelerate the rate of peptide hydrolysis.

(vii) liquid junction potentials are much more significant at this lower concentration [58,64].

(viii) many metal ion hydrolysis constants have been reported [65] for 25°C, 3.00M$NaClO_4$ but relatively few for 37°C, 0.150M$NaClO_4$. This means that ion hydrolysis studies may be necessary before further complexing reactions can be considered.

Clearly, there is no ideal medium, each set of conditions having certain merits and problems. In this work 25°C, 3.00M$NaClO_4$ was used so that comparison could be made to earlier results from this laboratory.

Formation Curves

The degree of formation of a system, $\bar{Z}$, was defined by Bjerrum [23] as the average number of ligands attached to the central group.

Formation curves are plots of $\bar{Z}$ *versus* -log a.

Consider the following system of stepwise equilibria forming mononuclear species only

$$A + B \rightleftharpoons AB$$

$$AB + A \rightleftharpoons A_2B$$

$$\vdots$$

$$A_{p-1}B + A \rightleftharpoons A_pB$$

Now $\bar{Z}$ $= \dfrac{[\text{ligand bound to central group}]}{[\text{central group}]}$ (5)

$$= \frac{[AB] + 2[A_2B]+.....+P[A_pB]}{[B] + [AB] + [A_2B]+....+[A_pB]} \quad (6)$$

$$= \frac{\sum_{1}^{P} p\,[A_pB]}{\sum_{o}^{P} [A_pB]} \quad (7)$$

β_p is defined as before i.e. $\beta_p = \dfrac{[A_pB]}{[A]^p\,[B]}$

Thus $[A_pB] = \beta_p[A]^p[B]$

and so $\bar{Z}$ $= \dfrac{\sum_{1}^{P} p\beta_p[A]^p[B]}{\sum_{o}^{P} \beta_p[A]^p[B]}$ (8)

$$= \frac{\sum_{1}^{P} p\,\beta_p[A]^p}{\sum_{o}^{P} \beta_p[A]^p} \quad (9)$$

From equation (9) we see that $\bar{Z}$ is a function of the concentration of free A, a, only and so formation curves determined at different total metal concentrations should be identical provided that only mononuclear complexes are formed. However, if polynuclear species are present then $\bar{Z}$ also depends on b [56].

Bjerrum's $\bar{Z}_{\frac{1}{2}}$ Method

Approximate formation constants, for mononuclear systems, may be estimated from formation curves using Bjerrum's $\bar{Z}_{\frac{1}{2}}$ method [23].

As before $\beta_p = \dfrac{[A_pB]}{[A]^p\,[B]}$ and $\beta_{p-1} = \dfrac{[A_{p-1}B]}{[A]^{p-1}\,[B]}$

and so $K_p = \dfrac{\beta_p}{\beta_{p-1}} = \dfrac{[A_pB]}{[A_{p-1}B][A]}$ (10)

therefore

$$\log K_p = \log [A_pB] - \log[A_{p-1}B] - \log a \qquad (11)$$

At $\bar{Z} = p-0.5$; $[A_pB] \simeq [A_{p-1}B]$

and so from (11)

$$\log K_p \simeq -\log a$$

The approximate formation constants thus determined can be used as initial estimates for computer refinement.

Determination of Experimental Formation Curves

Before beginning the experimental determination of the metal complexation constants of a ligand we determine the value of its protonation constants. Thus we have equilibrium constants for the reactions

$$H + A \rightleftharpoons HA \qquad \beta_1$$

$$2H + A \rightleftharpoons H_2A \qquad \beta_2$$

$$RH + A \rightleftharpoons H_RA \qquad \beta_R$$

Now in the simple metal complexing system we have the mass-balance relationship

$$\text{Total } H = T_H = h - \frac{w_k}{h} + [HA] + 2[H_2A] + \ldots\ldots + R[H_RA] \tag{12}$$

$$= h - \frac{w_k}{H} + \sum_1^R r\,[H_rA] \tag{13}$$

$$\text{Thus } T_H - h + \frac{w_k}{h} = \sum_1^R r\,[H_rA] = \sum_1^R r\beta_r h^r a \tag{14}$$

$$\text{and so} \qquad a = \frac{T_H - h + \frac{w_k}{h}}{\sum_1^R r\beta_r h^r} \tag{15}$$

$$\text{and} \qquad pa = -\log \left[\frac{T_H - h + \frac{w_k}{h}}{\sum_1^R r\beta_r h^r} \right] \tag{16}$$

Also Total A = T_A = a + [HA] + $[H_2A]$+....+$[H_RA]$ + [ligand bound to metal] (17

$$= a + \sum_{1}^{R} [H_rA] + [\text{ligand bound to metal}] \quad (18)$$

and so [ligand bound to metal] = $T_A - a - \sum_{1}^{R} [H_rA]$ (19)

Thus from equation (5)

$$\bar{Z} = \frac{T_A - a - \sum_{1}^{R} [H_rA]}{T_B} \quad (20)$$

$$= \frac{T_A - a - \sum_{1}^{R} \beta_r h^r a}{T_B} \quad (21)$$

$$= \frac{T_A - \left[\frac{T_H - h + \frac{w_k}{h}}{\sum_{1}^{R} r\beta_r h^r}\right] (1 + \sum_{1}^{R} \beta_r h^r)}{T_B} \quad (22)$$

Thus $\bar{Z}$ and pa can be calculated and the formation curve drawn provided that we know the ligand protonation constants, the ionic product of water, the total concentrations of A, B and H and the concentration of free hydrogen ion. The measurement of this free hydrogen ion concentration is the essence of the potentiometric approach to stability constant determination.

The Nernst Equation

An electrode in contact with the equilibrium mixture

$$kK + lL + ze \rightleftharpoons mM + nN \tag{23}$$

will develop the potential given by the Nernst equation[66]

$$E = {}^{T}E_{o} + \frac{RT}{zF} \ln \frac{(K)^{k} (L)^{l}}{(M)^{m} (N)^{n}} \tag{24}$$

where the standard electrode potential, ${}^{T}E_{o}$, is the potential acquired when all the species are at unit activity. However, if the activity coefficients are held constant equation (24) becomes

$$E = E_{o} + \frac{RT}{zF} \ln \frac{[K]^{k} [L]^{l}}{[M]^{m} [N]^{n}} \tag{25}$$

where the formal electrode potential, E_{o}, is given by

$$E_{o} = {}^{T}E_{o} + \frac{RT}{zF} \ln \frac{f_{K}^{k} f_{L}^{l}}{f_{M}^{m} f_{N}^{n}} \tag{26}$$

For the glass electrode equation (25) reduces to

$$E = E_{o} + \frac{RT}{F} \ln h \tag{27}$$

and so in a constant ionic medium h can be determined from the potential of the glass electrode.

Thermodynamic Functions

For an isothermal process the Gibbs free energy of reaction, $\Delta G^{\ominus}$, is related to the stability constant by the reaction isotherm[66]

$$\Delta G^{\ominus} = -RT \ln\beta \qquad (28)$$

The enthalpy of reaction, $\Delta H^{\ominus}$, may be measured calorimetrically or calculated from the temperature variation of the stability constant by use of the reaction isochore [66]

$$\left(\frac{\partial \ln\beta}{\partial T}\right)_p = \frac{\Delta H^{\ominus}}{RT^2} \qquad (29)$$

which on integration gives

$$\ln \frac{\beta_2}{\beta_1} = \frac{-\Delta H^{\ominus}}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \qquad (30)$$

if we assume that $\Delta H^{\ominus}$ does not vary with T. This assumption may often be invalid, for example see reference 67.

The entropy of reaction, $\Delta S^{\ominus}$, can now be calculated by use of the Gibbs-Helmholtz equation [66]

$$\Delta G^{\ominus} = \Delta H^{\ominus} - T\Delta S^{\ominus} \qquad (31)$$

As we can see $\Delta G^{\ominus}$, the value of which determines whether a reaction will proceed, depends on the balance of $\Delta H^{\ominus}$ and $\Delta S^{\ominus}$ with $\Delta H^{\ominus} < 0$ and/or $\Delta S^{\ominus} > 0$ favouring reaction.

CHAPTER 3

COMPUTATIONAL ASPECTS

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CHAPTER 3

COMPUTATIONAL ASPECTS

The mathematical treatment of experimental data in many branches of chemistry has been greatly aided by the development of electronic digital computers with adequate storage capacities and fast computing times. Indeed, the use of computers allows systems of greatly increased complexity to be studied. These computations have been the subject of several reviews [68,69].

The computer programs used in this work are written in FORTRAN IV and are loaded on to the St. Andrews University Computing Laboratory's IBM 360/44 computer.

Potentiometry

Having obtained potentiometric data for a metal ligand system, our aim is to completely define the system in terms of formation constants for all the complexes present. Since Bjerrum's pioneering work on metal ammine formation [23], the methods of choice for evaluating these constants have used relationships

involving the formation function, $\bar{Z}$, and the free ligand concentration.

It is possible to compute formation constants directly from the potentiometric data, however, there is a risk that, in this way, systematic errors or, indeed, some complex species, may be overlooked and erroneous conclusions drawn [70,71]. Thus we first of all prefer to obtain a plotted formation curve i.e. a plot of $\bar{Z}_h$ *versus* ph for a ligand-proton system or of $\bar{Z}$ *versus* pa for a ligand-proton-metal system. These curves can readily be used to pick out faulty data, for example an analytical or a card punching error may produce a grossly displaced curve.

ZPLOT [72,73]

The ZPLOT program, the mathematics of which have been described in Chapter 2, will plot either $\bar{Z}_h$ *versus* ph or $\bar{Z}$ *versus* pa curves. It is important to note that the mass balance relations in this program assume mononuclearity and the absence of hydroxy and protonated complexes. If these conditions are not valid then the calculated $\bar{Z}$ and pa are really pseudo $\bar{Z}$ and pseudo pa these functions being ideal for showing the degree of variation from mononuclearity etc. Under these conditions the curves may form a complicated pattern. Although various plots have been used to simplify these patterns, for example, Österberg plotted $Y[=(T_H-h)/A]$ against ph, [74] the selection of species to describe these curves is not a simple matter. We have both the qualitative problem of deciding which complexes are present and the quantitative problem

of determining their formation constants. Qualitatively the possible complexes are limited by the coordination numbers of the metal ion and by the denticity of the ligand [32]. However, as will be discussed later in this chapter, the pattern of the formation curves may also give some indication of species present.

Many computer programs of varying complexity have been written for the determination of formation constants [68], for all but the very simple systems a non-linear least-squares approach being necessary. Several programs of this type have been described in the literature e.g. SCOGS [75], LETAGROPVRID [71,76-78], and MINIQUAD [79].

Both SCOGS and MINIQUAD have been used in this work in the hope that there are sufficient mathematical differences between these programs to highlight spurious formation constants that may have occurred had just one program been used. Formation constants produced from the two programs generally agreed to within the standard deviation.

SCOGS

Our version of the program SCOGS (Stability Constants Of Generalised Species) has been slightly modified from the published version [80,81,82]. It can deal with a system of two ligands, two metals, up to twenty complexes, up to thirty titrations, up to five-hundred data points, up to three ion-selective electrodes and can refine constants for polynuclear, hydroxy, protonated or mixed

complexes provided that the degree of complex formation is ph dependent.

The Newton-Raphson method of iteration is used to minimise $U = \sum_i (\text{titre}^i_{\text{expt.}} - \text{titre}^i_{\text{calc.}})^2$ where $\text{titre}_{\text{calc.}}$ is calculated from the current estimates of the formation constants and all the experimental values of ph. If the value of any constant is accurately known it may be retained at this value during the calculation. If, on the other hand, any complex species is introduced which is inconsistent with the data, then it is impossible to obtain a converging value for its formation constant which progressively diminishes while the concentration of the species becomes negligible.

The estimated standard deviations in the computed formation constants indicate the error limits for each constant and the standard deviation in titre is a measure of the ability of the computed constants to reproduce the experimental titrations. SCOGS has been shown to have certain mathematical defects which may cause it to fail to converge to satisfactory solutions [83]. Also, for some complex systems, it was found to be impossible to refine all the constants together because of the occurrence of 'exponent overflow'. This problem can be circumvented by the use of MINIQUAD.

MINIQUAD

MINIQUAD (from the Italian for least-squares, minimi quadrati) can, in principle, deal with data from systems containing any number of reactant species and potentiometric electrodes and all commonly found types of complex.

The Gauss-Newton method of refinement, which has been shown [84] to be preferable to the Newton-Raphson method, is used to minimise the sum of squared residuals for all the mass balance equations, $U = \sum_i (T^i_{calc.} - T^i_{obs.})^2$. The least-squares minima are approached using the Davidon-Fletcher-Powell steepest descent method rather than a Jacobian matrix.

Formation constants are stored in mantissa and exponent form, only the mantissa being varied during the refinement so allowing formation constants of any magnitude to be determined regardless of the numerical capacity of the computer. In reference 80, nine advantages of MINIQUAD over other programs, such as SCOGS are listed.

Finally a statistical analysis of results is made in order to assess their validity and to assist in hypothesis testing.

Altough least-squares programs, such as SCOGS and MINIQUAD, produce statistical analyses or numerical values of standard deviations in titre or sums of squared residuals the human mind prefers to see differences between calculated and experimental data in diagrammatic form. If any discrepancies are then found one may be able to suggest an improvement in the chemical model. As has been pointed out, [69] "the observably good fit of a back-calculated curve to a set of data is an effective justification

of a set of stability constants, and presents evidence to an experimental chemist in a way that tables of residuals and standard deviations do not".

Our work on the lead(II)-glycylglycinate system produced the formation curves shown in figure 3 which indicate that other than simple mononuclear species are present. The program HALTAFALL [85], using input formation constants for the species 01-1,* 04-4, 03-4, 06-8, 110, and 111 as well as the ligand protonation constants, was used to calculate the concentrations of species present at each point in the titration. From these concentrations the program can calculate theoretical volumes of titrant added and emfs which can then be used as input for ZPLOT to give a calculated pseudo $\bar{Z}$ *versus* pseudo pa plot. If the formation constants input to HALTAFALL completely describe the system then the curves obtained in this way should be an exact replica of the experimental formation curves. The simulated curves for the lead(II)-glycylglycinate system are shown in figure 4.

Thus we obtain a graphical representation of how well the calculated formation constants can reproduce the experimental data. It was thus thought worthwhile to combine the programs HALTAFALL and ZPLOT to produce the new program PSEUDOPLOT [61,86] which will perform the above process in a single step.

* The species $A_p B_q H_r$ is represented by pqr where r < 0 indicates a hydroxyl group or the removal of a proton.

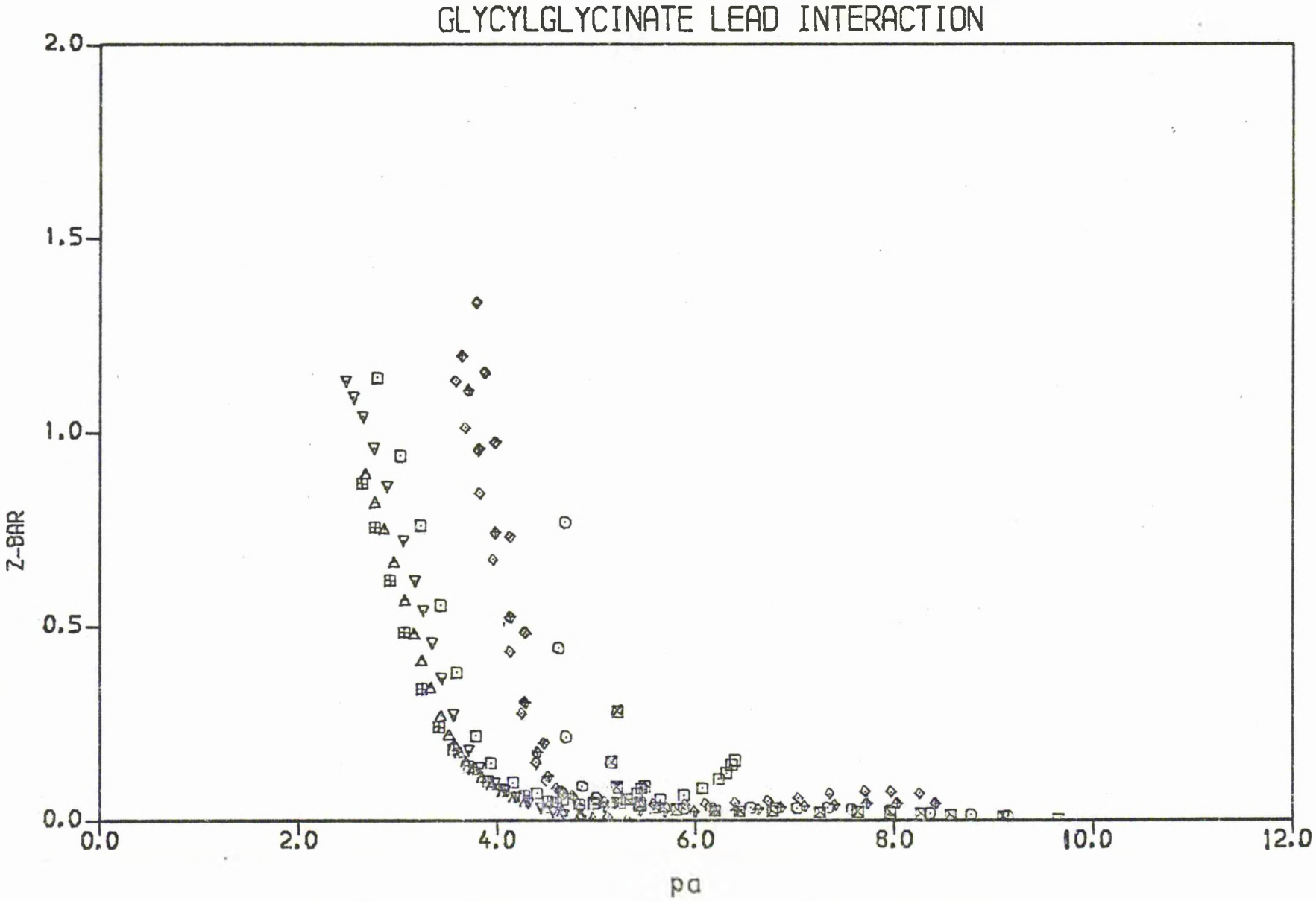

FIGURE 3 : EXPERIMENTAL CURVES FOR THE LEAD(II)-GLYCYLGLYCINATE SYSTEM

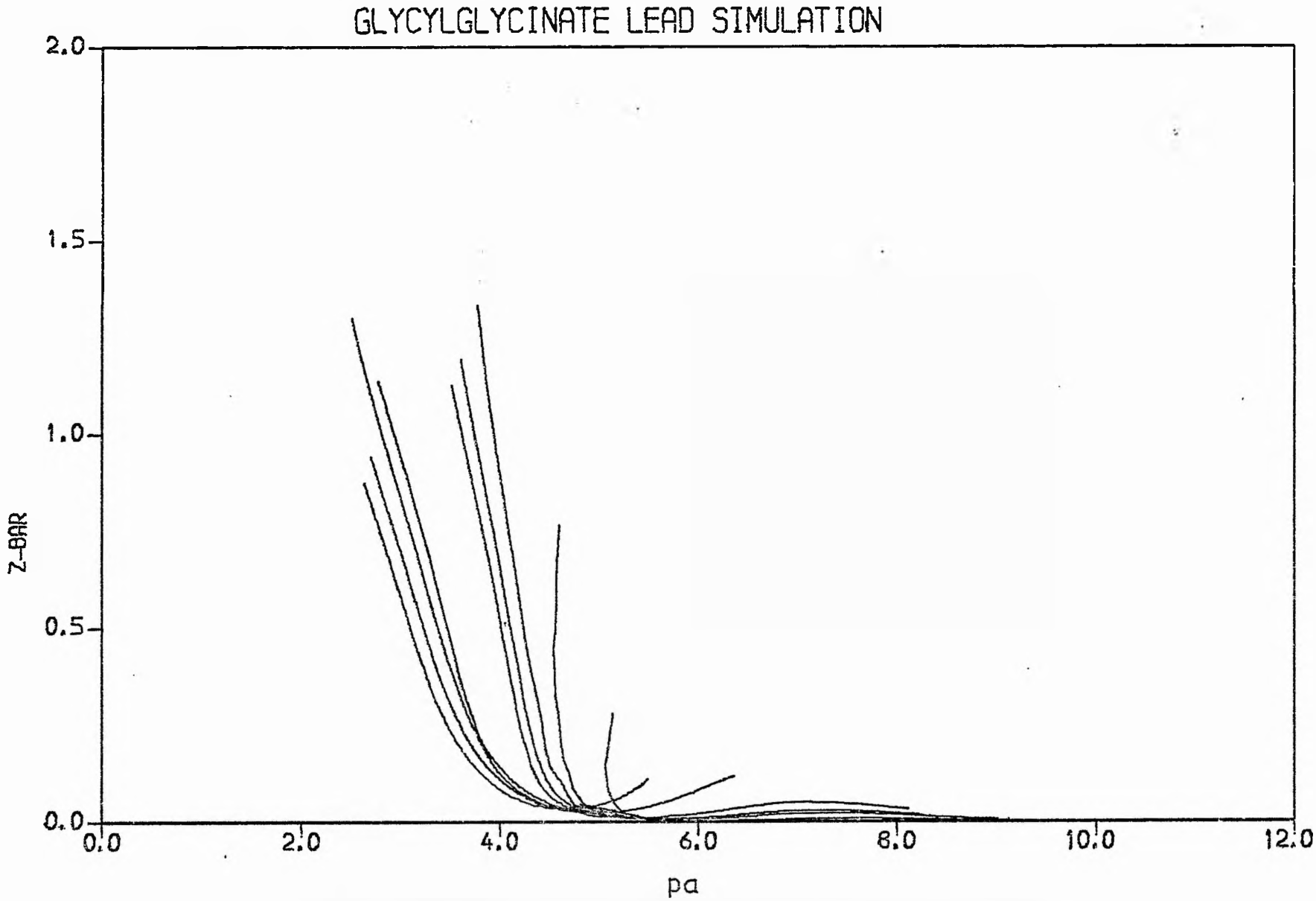

FIGURE 4 : SIMULATED CURVES FOR THE LEAD(II)-GLYCYLGLYCINATE SYSTEM

HALTAFALL

HALTAFALL (from the Swedish 'halt' = concentration and 'falla' = precipitate) calculates the equilibrium concentrations of the species present in a mixture of any number of components, which can form any number of complexes and solid phases, provided that the equilibrium constants are known and enough information is given about the gross composition of the system.

PSEUDOPLOT

In PSEUDOPLOT, the input formats have been greatly simplified from the original HALTAFALL. The HALTAFALL section has as input the experimental conditions (i.e. concentrations in titrate and titrant and volumes etc. (all except the emf or ph readings)) and the postulated formation constants. The output is simulated volume added *versus* ph data that would have been obtained had such a system been titrated experimentally. The program then uses this data in the ZPLOT section to produce simulated titration curves which can then be compared with the experimental curves and additional sets of formation constants can be tried until the 'best' fit is obtained.

Thus PSEUDOPLOT can provide an alternative to graphical normalised curve fitting [56] as a means of detecting non-simple complexes.

Normalised, or standardised, curves are projection maps of model functions calculated on the assumption that certain species

coexist at equilibrium (as defined by a set of formation constants) and these can be compared with plots of experimental data covering a wide range of concentrations. In this way it is possible to discover which species are probably present (and the position of 'best' fit reveals the values of their formation constants). Alternative sets of formation constants ought always to be tested but, unfortunately, each set necessitates the calculation of a unique pattern of normalised curves, each pattern being based on different mathematical relations [87]. For example, for the system in which only AH, A_2H and A_2H_2 are postulated, the normalised curves are calculated from

$$\underline{T_A} = \frac{[\underline{h} - (1 + \underline{h})\ \bar{Z}_h]\ [\underline{h}(1 - 2R) - 1]}{\underline{h}[2\bar{Z}_h\ (1 + R\underline{h}) - (1 + 2R\underline{h})]^2}$$

where $R = \frac{(2\bar{Z}_h - 1)}{2\bar{Z}_h}$ and $\underline{T_A}$ and $\underline{h}$ are normalised variables corresponding to T_A and h[88,89]. This represents the case for a fairly simple system but when more than three formation constants are introduced the mathematics becomes much more complicated [56,90].

Graphically PSEUDOPLOT is equivalent to the normalised curves method but by using a common series of relations (as distinct from a different series of equations for each set of formation constants) and a graph plotter it is much faster.

By observing the regions of mismatch between the ZPLOT and PSEUDOPLOT curves it may be possible to suggest a complex whose presence would improve the fit. For example, the 'curl-back'

sometimes found at the high $\bar{Z}$ end of formation curves may be reproduced by the introduction of a hydroxy species and the '$\bar{Z}$-hump' sometimes found at high pa by a protonated species.

By trial of many sets of formation constants we can thus find the set which gives the 'best' fit to the experimental data. We must remember, however, that the 'best' set of formation constants need not be the 'right' set and a set of constants, no matter how they are obtained, can never be more than 'compatible with the available experimental data'.

Having obtained our 'best' set of formation constants we may now wish to know which of the complexes predominates at any given pH or we may wish to incorporate the constants into a model of a biological system. Either of these objectives may be achieved by the use of our program COMPLOT [91].

COMPLOT

COMPLOT is a modified version of the program COMICS [92] which can deal with up to ten metals, ten ligands, one-hundred complexes and fifty pH values and is applicable in the presence of all common types of metal complex.

The basis of this program and, of course, all other equilibrium programs is the law of mass action and it can be used to calculate

the equilibrium concentrations of all species in a multi-metal-multi-ligand mixture, given the pH of the solution, the total concentration of each metal and ligand and the relevant formation constants.

If from the computational analysis the presence of a particular complex is still doubtful then a COMPLOT model can show whether it would be present in significant quantities under the titration conditions.

Calorimetry

Before the calorimetric work is begun the systems have been studied potentiometrically and so the relevant formation constants are already known. Thus PSEUDOPLOT can be used to calculate the concentrations of species present for each point in the calorimetric titrations. The program CALCO may then be used to apply corrections to the measured heat changes in order to account for the heats of formation of water, of hydrolysed metal species and of protonated ligand species, as appropriate. The corrected heat changes are then used along with the changes in concentration of each species in order to calculate enthalpies of formation of the complexes.

CALCO

CALCO is an extended version of the program CALCRD [81] and can deal with four protonated ligand and four metal-hydroxy species.

The species concentrations from PSEUDOPLOT are used to calculate the heat corrections mentioned above and the change in the number of moles (Δn_m) of each complex present for each point in the titration. Thus the following equations for the m complexes are set up

$$(Q_{corr})_1 = (\Delta H_1^{\ominus} \Delta n_1)_1 + (\Delta H_2^{\ominus} \Delta n_2)_1 + \ldots\ldots\ldots + (\Delta H_m^{\ominus} \Delta n_m)_1$$

$$(Q_{corr})_2 = (\Delta H_1^{\ominus} \Delta n_1)_2 + (\Delta H_2^{\ominus} \Delta n_2)_2 + \ldots\ldots\ldots + (\Delta H_m^{\ominus} \Delta n_m)_2$$

and so on to

$$(Q_{corr})_r = (\Delta H_1^{\ominus} \Delta n_1)_r + (\Delta H_2^{\ominus} \Delta n_2)_r + \ldots\ldots\ldots + (\Delta H_m^{\ominus} \Delta n_m)_r$$

for the r titration points.

Normally r >> m and so a least-squares set of $\Delta H_m^{\ominus}$ values can be computed by minimising

$$U = \sum_1^r (Q^{\,i}_{corr.} - Q^{\,i}_{calc.})^2$$

CHAPTER 4

REAGENTS AND APPARATUS

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CHAPTER 4

REAGENTS AND APPARATUS

Reagents

The reagents used in this work were prepared and analysed as follows.

WATER

All solutions were prepared using 'Elgastat' (The Elga Group) deionised water the resistivity of which was higher than 2MΩcm.

SODIUM PERCHLORATE

Solutions of sodium perchlorate were made either by dissolving the monohydrate(Merck 'Puriss', B.D.H.AnalaR or Hopkin and Williams AnalaR) in water, or by neutralising perchloric acid(Fisons A.R.) with sodium carbonate(Fisons A.R.). The pH of the solution was adjusted to 9-10 by addition of sodium hydroxide solution(B.D.H. AnalaR) before allowing it to stand for at least 7 days. The precipitate

of silica and heavy metal oxides and hydroxides was then removed by filtration through micropore filters of pore diameters 3.0 and 0.45μm(Millipore Ltd.). The solution was then acidified to pH 2 by the addition of perchloric acid and the carbon dioxide was removed by boiling and cooling under nitrogen.

From this point one of two alternative methods was used.

(i) Crystals were prepared from the above solution by adjusting its pH to 7 and heating in an evaporating basin to 140°C. After cooling to 105°C, the slurry was filtered through a sintered glass funnel (porosity 3) and dried in an oven at 110°C.

(ii) A standard solution was prepared by adjusting the pH to 7 and analysing by cation exchange[93a] and flame photometry [93b].

PERCHLORIC ACID

A stock solution of perchloric acid (~3M) was prepared by dilution of concentrated perchloric acid (60% w/w, Fisons A.R.). This was standardised by titration with sodium carbonate, which had been heated at 260-270°C for 30 minutes and dried in a desiccator, using methyl orange as indicator [93c] and checked against standard sodium hydroxide solution [93d].

SODIUM HYDROXIDE

1.00M and 0.100M solutions of sodium hydroxide were prepared from ampoules(B.D.H. concentrated volumetric solutions) and were checked against standard acid solution [93d].

SODIUM CHLORIDE

Sodium chloride(Fisons A.R.) was dried at 200°C before use.

HYDROCHLORIC ACID

1.00M solutions of hydrochloric acid were prepared from ampoules(B.D.H. concentrated volumetric solutions) and were checked against standard sodium hydroxide solution [93d].

METAL ION SOLUTIONS

All metal ion solutions were prepared and analysed by two independent methods.

(i) Lead Perchlorate

Lead oxide(PbO, Hopkin and Williams) was dissolved in perchloric acid(60%), allowed to stand for several days and

filtered through a micropore filter(0.45μm). Analysis was by edta titration, with xylenol orange as indicator [93e], and by electrodeposition [93f].

(ii) Zinc Perchlorate

Zinc perchlorate(G.F. Smith, Chemical Co.) was dissolved in perchloric acid, allowed to stand for several days and filtered through a micropore filter(0.45μm). Analysis was by edta titration, with eriochrome black T as indicator [93g], and gravimetrically as quinaldinate [93h].

(iii) Nickel Perchlorate

Nickel perchlorate(G.F. Smith, Chemical Co.) was dissolved in perchloric acid, allowed to stand for several days and filtered through a micropore filter(0.45μm). Analysis was by edta titration, with murexide as indicator [93i], and by electrodeposition [93j].

(iv) Nickel Chloride

Nickel chloride(B.D.H. AnalaR) was dissolved in hydrochloric acid(1M), allowed to stand for several days and filtered through a micropore filter(0.45μm). Analysis was by edta titration, with murexide as indicator [93i], and by electrodeposition [93j].

(v) Copper Chloride

Copper oxide(Hopkin and Williams AnalaR) was dissolved in hydrochloric acid(35%), allowed to stand for several days and filtered through a micropore filter(0.45μm). Analysis was by edta titration, with fast sulphon black F as indicator [93k], and by electrodeposition [93l].

The hydrogen ion concentration of each of these solutions was obtained by means of Gran plots [94].

ETHYLENEDIAMINETETRAACETATE

The disodium salt of ethylenediaminetetraacetic acid(B.D.H. AnalaR) is a primary standard and, therefore, solutions were prepared by direct weighing [93m].

LIGANDS

The following ligands were used without further purification.

(i) Aspartic acid(B.D.H. Biochemicals) m.p. decomposes at> 210°C; lit. 271°C. Found C, 35.9; H, 5.4; N, 10.5%. Calc. for $C_4H_7NO_4$ C, 36.1; H, 5.3; N, 10.5%.

(ii) Cysteine(E. Merck A.G.) m.p. decomposes 179°C; lit. 175-178°C decomp. Found C, 29.8; H, 5.9; N, 11.4%. Calc for $C_3H_7NO_2S$ C, 29.7; H, 5.8; N, 11.6%.

(iii) Glutamic acid(B.D.H. Biochemicals) m.p. 197-199°C; lit. 224-225°C. Found C, 40.8; H, 6.3; N, 9.5% Calc. for $C_5H_9NO_4$ C, 40.9; H, 6.2; N, 9.5%

(iv) Glycine(Fisons A.R.) m.p. decomposes 244°C; lit. 262°C decomp. Found C, 32.1; H, 6.5; N, 18.5%. Calc. for $C_2H_5NO_2$ C, 32.0; H, 6.7; N, 18.6%.

(v) Glycylglycine(Koch Light) m.p. decomposes 250°C; lit. 260-262°C decomp. Found C, 36.3; H, 6.4; N, 21.1%. Calc. for $C_4H_8N_2O_3$ C, 36.4; H, 6.1; N, 21.2%.

(vi) Glycylglycylglycine(Koch Light) m.p. decomposes 236°C; lit. 246°C decomp. Found C, 38.1; H, 5.9; N, 22.3%. Calc. for $C_6H_{11}N_3O_4$ C, 38.1; H, 5.9; N, 22.3%.

(vii) Glutathione(Sigma) m.p. decomposes 192-194°C; lit. 190-192°C decomp. Found C, 38.8; H, 5.8; N, 13.5%. Calc. for $C_{10}H_{17}N_3O_6S$ C, 39.1; H, 5.6; N, 13.7%.

(viii) Disodium ethylenediaminetetraacetate(B.D.H. AnalaR) m.p. decomposes 246°C; Found C, 32.5; H, 5.2; N, 7.5%. Calc. for $C_{10}H_{18}N_2Na_2O_{10}$ C, 32.3; H, 4.9; N, 7.5%.

(ix) D-penicillamine(Koch Light) m.p. decomposes 209°C; lit. 203°C decomp. Found C, 40.3; H, 7.8; N, 9.2%. Calc. for $C_5H_{11}NO_2S$ C, 40.2; H, 7.4; N, 9.4%.

(x) Bovine serum albumin(B.D.H. Biochemicals). Found C, 47.9; H, 6.7; N, 14.8%. Calc. for $C_{2906}H_{4749}N_{778}O_{883}S_{39}$ [96] C, 52.9; H, 7.3; N, 16.5%.

(xi) Histidine(Koch Light) m.p. decomposes 250°C; lit. 270-280°C. Found C, 46.2; H, 5.9; N, 27.1%. Calc. for $C_6H_9N_3O_2$ C, 46.5; H, 5.9; N, 27.1%.

(xii) Threonine(B.D.H. Biochemicals) m.p. decomposes 245°C; lit. 251-253°C. Found C, 40.5; H, 7.8; N, 11.6%. Calc. for $C_4H_9NO_3$ C, 40.3; H, 7.6; N, 11.8%.

(xiii) Aspirin(Lilly Research Centre Ltd.) m.p. 128-132°C; lit. 135°C. Found C, 60.1; H, 4.4%. Calc. for $C_9H_8O_4$ C, 60.0; H, 4.5%.

(xiv) Salicylic acid(B.D.H. AnalaR) m.p. 156-157°C; lit. 159°C. Found C, 60.8; H, 4.3%. Calc. for $C_7H_6O_3$ C, 60.9; H, 4.4%.

(xv) Indomethacin(Merck, Sharp and Dohme) m.p. 154-155°C; lit. 153-154°C. Found C, 63.8; H, 4.4; N, 3.7%. Calc. for $C_{18}H_{14}NO_4Cl$ C, 63.8; H, 4.5; N, 3.9%.

(xvi) Naproxen(Syntex Labs.) m.p. 150-152°C; Found C, 73.1; H, 6.1%. Calc. for $C_{14}H_{14}O_3$ C, 73.0; H, 6.1%.

(xvii) Ketoprofen(May and Baker Ltd.) m.p. 91-93°C; Found C, 73.0; H, 6.0%. Calc. for $C_{16}H_{14}O_3$ C, 75.6; H, 5.6%.

(xviii) Calcium fenoprofen(Lilly Research Centre Ltd.) m.p. 120-124°C; Found C, 64.5; H, 5.4%. Calc. for $C_{30}H_{26}O_6Ca$ C, 69.0; H, 5.0%.

(xix) Phenylbutazone(Lilly Research Centre Ltd.) m.p. 103-104°C; lit. 105°C. Found C, 74.1; H, 6.5; N, 9.0%. Calc. for $C_{19}H_{20}N_2O_2$ C, 74.0; H, 6.5; N, 9.1%.

NITROGEN

Oxygen free nitrogen(British Oxygen Company) was bubbled through 3.00M sodium perchlorate solution thermostatted at 25°C before use.

Apparatus

GLASSWARE

All pipettes and volumetric flasks(Technico Grade A) were provided with calibration certificates. All glassware was cleaned regularly with Quadralene(Quadralene Chemical Products) and with alcoholic potassium hydroxide if required.

POTENTIOMETRY

Potentiometric studies were carried out using the vessel shown in figure 5(Pye Ingold 604) the working temperature being maintained by water from a thermostatted water bath (Grant Instruments). For work at 10°C a back-up cooler unit was used ('Paxman' Cooler Manufacturing Company).

The electrode pair(normally Russell pH Ltd.; glass SF 711/B14; calomel CR4/5/Na/B14) was used in conjunction with a digital voltmeter(Solartron LM1867) to give readings reproducible to 0.1mV. For the 'challenge project' work a silver/silver chloride

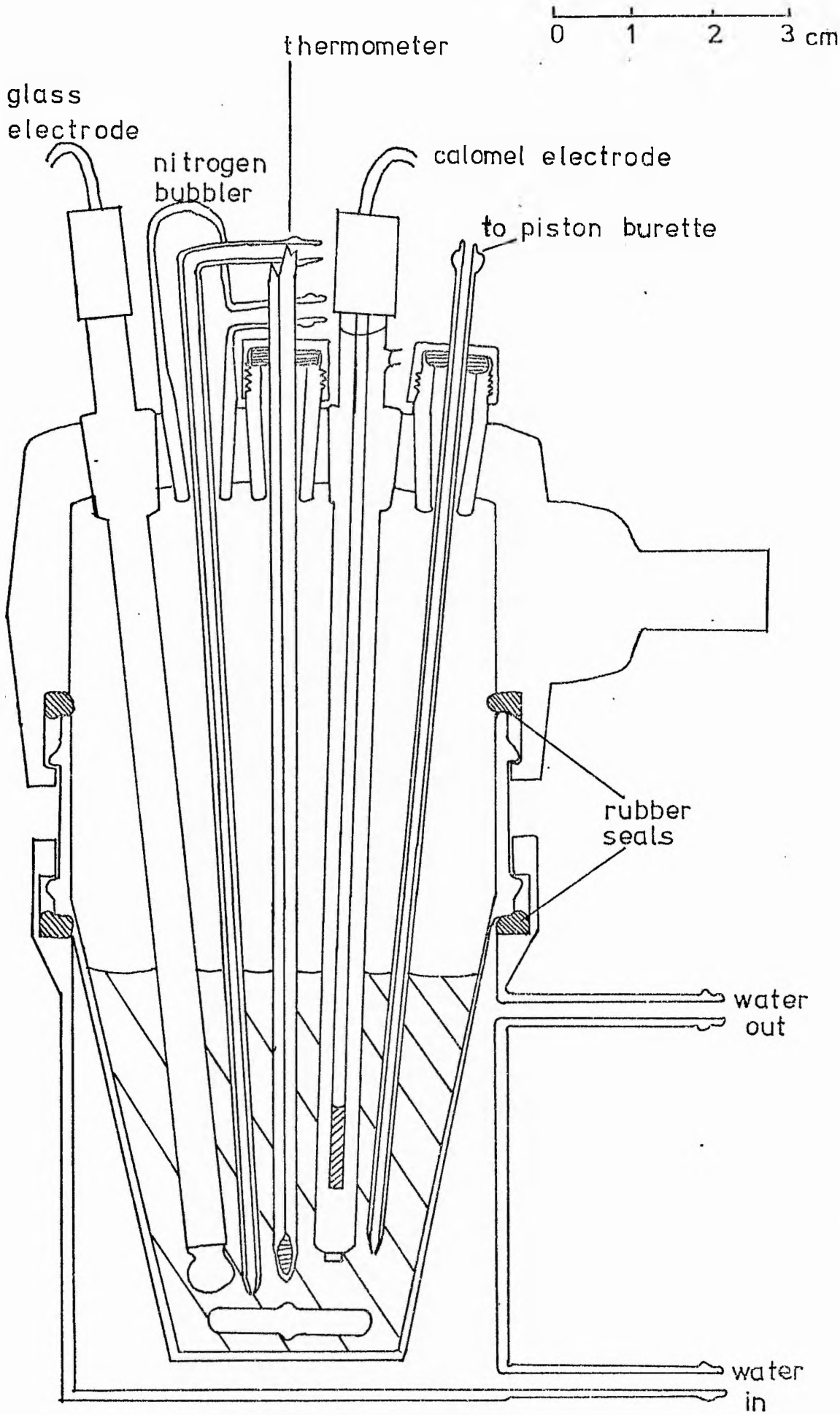


FIGURE 5 : POTENTIOMETRIC VESSEL

reference electrode was used(Russell pH Ltd.; UMA5) and was prepared by the method of reference 97. In this case the liquid junction was made through a closed ground glass tap. For the study of the interaction of chloride with lead(II) a glass electrode, a chloride sensitive electrode (prepared as above) and a silver/silver chloride reference electrode were used. The potential between the reference and the chloride sensitive electrode was measured on a digital voltmeter(Radiometer pHM52).

Titrant was added from a 10ml piston burette(Metrohm E274) and the solution was mixed by slow magnetic stirring.

CALORIMETRY

The calorimetric vessel, shown in figure 6, is of the Gerding, Leden and Sunner [97] constant environment type.

The complete system was suspended in a thermostat bath controlled to $\pm$ 0.0005°C(LKB 7602 controller on a 7603A bath) which was located in a thermostatted room (19 $\pm$ 0.5°C).

A glass inner reaction vessel was used and the inside of its lid was coated with epoxy resin(Bostic quick hardener).

Calibration was electrical using a heater coil of non-inductively wound resistance wire(22.38Ω) coated with epoxy resin(Araldite). The voltage across the heater was measured on a digital voltmeter(Solartron LM1420.2). The current flowing through the heater resistor also passed through a

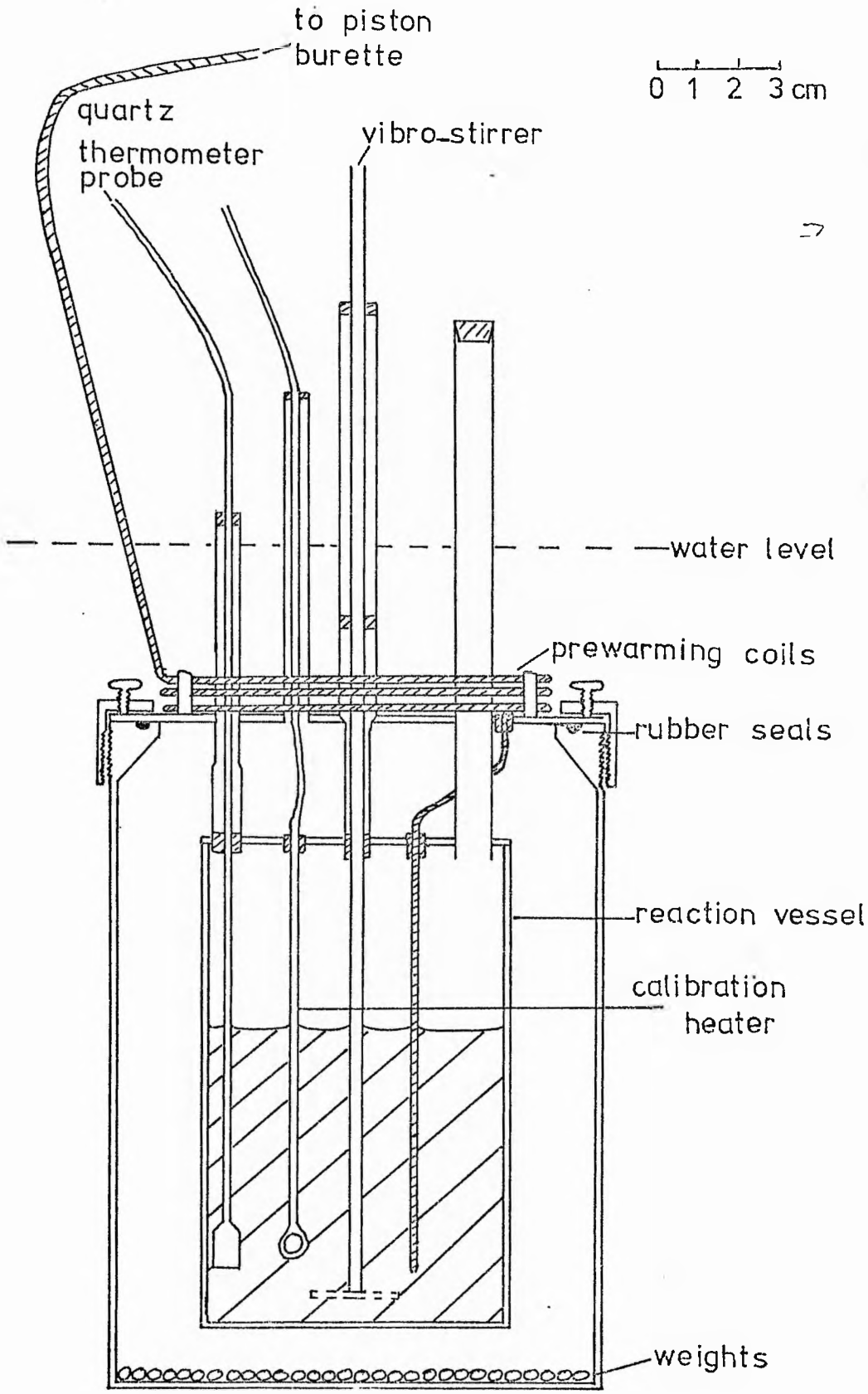


FIGURE 6 : CALORIMETRIC VESSEL

10.000Ω standard resistance and the voltage across this was measured to give the current in the circuit. The time for which the heating current flowed was recorded automatically to within 0.02s using a stopwatch(Jaquet 309e).

Vibro-stirring was used(Chemap AP, E1), the stirrer tube being hollow to allow insertion of an aluminium rod cooled in liquid nitrogen in order to lower the solution temperature between additions of titrant.

Titrant, from a 10ml piston burette(Metrohm E274), was prewarmed in a spiral of narrow bore nylon tubing(Portex SFD Nylon C) before being added to the reaction vessel through a glass burette tip.

The temperature change caused by the addition of the titrant, or by the calibration heater, was measured by a quartz thermometer(Hewlett-Packard 2801A) and was printed out(Hewlett-Packard digital recorder 562A) to the nearest 0.0001^{o}C at 18s intervals.

ULTRA-VIOLET/VISIBLE SPECTROSCOPY

90% ethanol solutions had their pHs adjusted by addition of 0.1M sodium hydroxide from an 'Agla' micrometer syringe (Burroughs Wellcome and Co.) before measurement of their spectra using a Pye Unicam SP 800B spectrophotometer. In the visible range 40mm glass cells were used and in the ultra-violet range 10mm silica cells(Pye Unicam).

MOLECULAR FILTRATION

The cell used for molecular filtration(Millipore Ltd., 25mm cell) is shown in figure 7.

A pressure of $2.8 \times 10^{5} Nm^{-2}$ was applied using oxygen free nitrogen.

The filters used(Millipore Ltd., PSED 25,000) have a nominal molecular weight cut-off of 25,000 and between uses were cleaned in 5% acetic acid and stored under water.

Solution pHs were adjusted by the addition of 0.1M sodium hydroxide from an 'Agla' micrometer syringe.

Analysis for copper was by atomic absorption spectrophotometry (Perkin-Elmer 360) using a multi-element lamp(Perkin-Elmer 303-6102) and a chart recorder(Perkin-Elmer Model 056) as well as a digital display(Perkin-Elmer UDR-3).

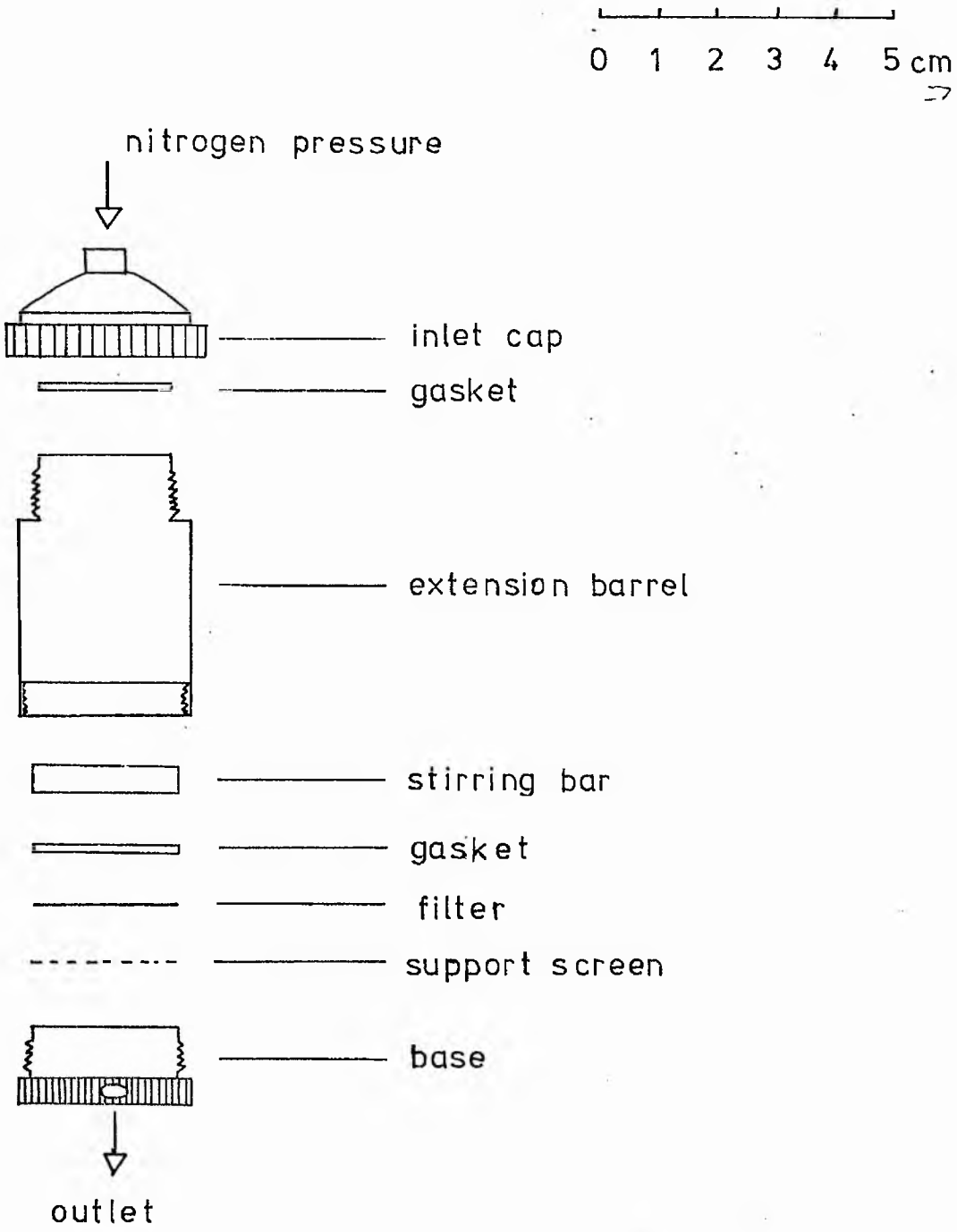


FIGURE 7 : MOLECULAR FILTRATION CELL

CHAPTER 5

RESULTS - FORMATION CONSTANTS

CONTENTS

CHAPTER 5

RESULTS - FORMATION CONSTANTS

All of the potentiometric experiments described in this chapter were carried out at 25°C, in an ionic background of 3.00M sodium perchlorate, using the apparatus described in Chapter 4.

The electrochemical cell used for the glass electrode work was

glass electrode	A, B and H solution under study, I = 3.00M(Na^+)ClO_4^-	NaCl (saturated)	Hg_2Cl_2(s)-Hg(l)

Electrode Calibration

As we have seen in Chapter 2, the above electrode pair gives a potential described by $E = E_o + \frac{RT}{F}\ln h$ and so, if E_o is constant, then measuring E for a series of solutions of known h and plotting E *versus* -log h ought to give a straight line of slope -2.303RT/F mV$(-\log h)^{-1}$. In practice, however, when an acid ionic background solution is titrated with an alkaline ionic background solution an S-shaped curve is obtained which indicates that traces of surplus base are being introduced from an unknown source. For the experimental data see table 1 and figure 8a (values of -log h > 7 were calculated using a value of -log W_k = 14.22)[98].

TABLE 1

Experimental data for the calibration of a glass/calomel electrode pair

Titrate : 20.01ml of 10.74mM $HClO_4$, I = 3.00M(Na^+)ClO_4^-

Titrant : 50.40mM NaOH, I = 3.00M(Na^+)ClO_4^-

Titre (ml)	E (mV)	-logh	Titre (ml)	E (mV)	-logh
0.20	334.6	1.99	4.31	-76.6	9.70
1.50	323.5	2.19	4.35	-116.1	10.27
2.70	307.8	2.45	4.39	-138.4	10.51
3.20	297.8	2.63	4.45	-160.7	10.72
3.60	285.3	2.83	4.55	-179.4	10.94
3.85	273.1	3.03	4.70	-194.5	11.13
4.00	261.7	3.21	4.90	-206.2	11.30
4.15	240.2	3.52	5.30	-220.2	11.52
4.21	221.5	3.75	5.90	-232.1	11.71
4.25	192.8	4.03	7.10	-246.0	11.93
4.27	149.4	4.28	8.50	-255.1	12.09
4.29	-16.3	4.97	10.00	-261.5	12.20

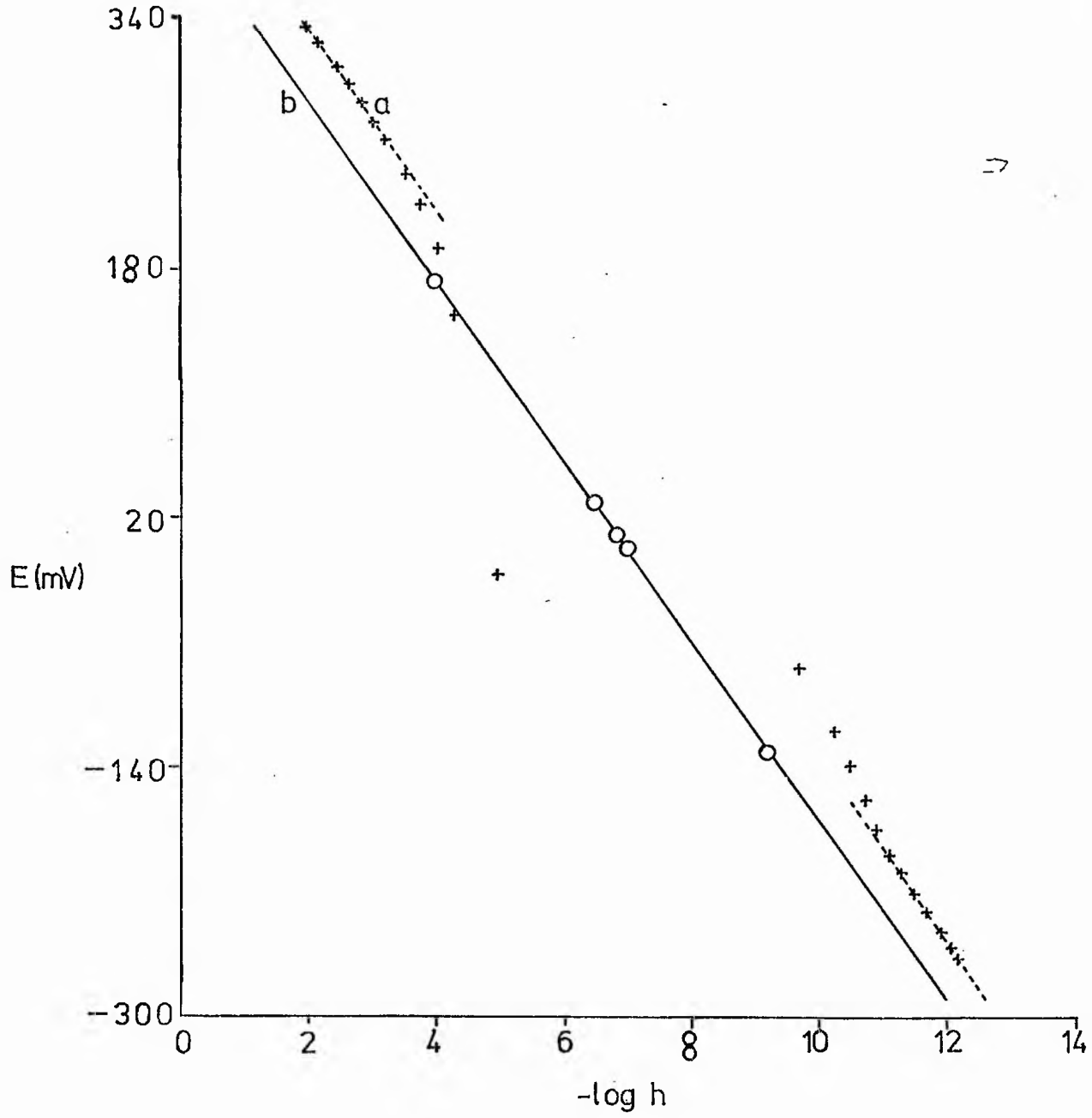


FIGURE 8 : GLASS/CALOMEL ELECTRODE PAIR CALIBRATION

a : CALIBRATION LINE b : BUFFER LINE

A straight line is obtained in the -log h ranges 2.0 - 3.5 and 11.0 - 12.2. It has been suggested [99] that the traces of alkali arise from slight dissolution of glass from the electrodes and the titration vessel ($\sim 5 \times 10^{-6}$ M alkali).

In buffered solutions, however, a linear response is found. For experimental data see table 2 and figure 8b.

TABLE 2

Experimental data for glass/calomel electrode pair response in buffered solutions

E (mV)	pH
171.2	4.00
29.1	6.50
8.0	6.865
-0.1	7.00
-129.7	9.20

All formation constant determinations take place in buffered solutions and so the glass interference is swamped by the buffering capacity of the system.

At the beginning of a series of titrations the electrodes are checked by the measurement of a buffer line. This line is in terms of activities, is not at constant ionic strength and is displaced

away from but parallel to the E *versus* -log h calibration line. The purpose of this buffer line is to choose the best of the available glass electrodes. A calibration line can then be constructed which is extrapolated to -log h = 0, using a simple least-squares procedure, to produce the best E_o value. This E_o value is checked before and after each titration by measuring E for a solution of known -log h (which must be in the range 2.0 - 3.5 because of the glass interference at other values).

Tolerances

In general all errors are kept to ≲0.1%. Some typical values are shown in table 3.

TABLE 3

Experimental errors

Stock solution concentrations	± 0.1%
Weights	± 0.05mg
Flasks	± 0.05%
Pipettes	± 0.1%
Piston burette	± 0.005ml
Solution temperature	± 0.05°C
E	± 0.1mV
E_o	± 0.2mV

Recalculation of Formation Constants from Reference 22

The work on lead(II) complexing to amino acid anions, reported in reference 22, was carried out before the program MINIQUAD was available or PSEUDOPLOT was devised. The data has thus been recalculated using our current approach to give the log formation constants shown in table 4. In each case the species $Pb(OH)^{+}$, $Pb_4(OH)_4^{4+}$, $Pb_3(OH)_4^{2+}$ and $Pb_6(OH)_8^{4+}$ with log formation constants of -7.9, -19.25, -22.87 and -42.14 [36] respectively were included in the computational analysis.

TABLE 4

Recalculated log formation constants for lead(II) - amino acid anion complexes at 25°C, I = 3.00M(Na^{+})ClO_4^{-}

	log β_{pqr}		
	110	210	310
Asparaginate	4.902 ± 0.018	7.802 ± 0.031	8.84 ± 0.28
Glutaminate	4.132 ± 0.086	7.08 ± 0.14	9.805± 0.084
Histidinate	6.901 ± 0.004	9.80 ± 0.10	
Phenylalaninate	4.314 ± 0.069	8.045 ± 0.060	
Serinate	4.949 ± 0.025	8.145 ± 0.046	9.873± 0.097
Tryptophanate	4.52 ± 0.29	9.58 ± 0.11	

It was decided that the data available for the lead(II) - aspartate and lead(II)-cysteinate systems was inadequate and so further work was done on these before the formation constants were recalculated.

Ligand Protonation Constants

For each ligand several experiments were performed, using different ligand concentrations, in which a solution of ligand and acid was titrated with a solution of alkali or of ligand and alkali.

All experimental data for the ligand protonation work is given in appendix 1 and the determined constants, with their standard deviations and the number of experimental observations, in table 5.

In general, protonation curves at different ligand concentrations are superimposable indicating the absence of polynuclear species. edta is an exception which will be discussed in the appropriate section.

GLUTAMATE

Experimental results are shown in appendix 1 - table 1 and in figure 9. (Plotted symbols represent titrations in the order shown on page 2.)

The computed log K values are 9.895, 4.518 and 2.572 the

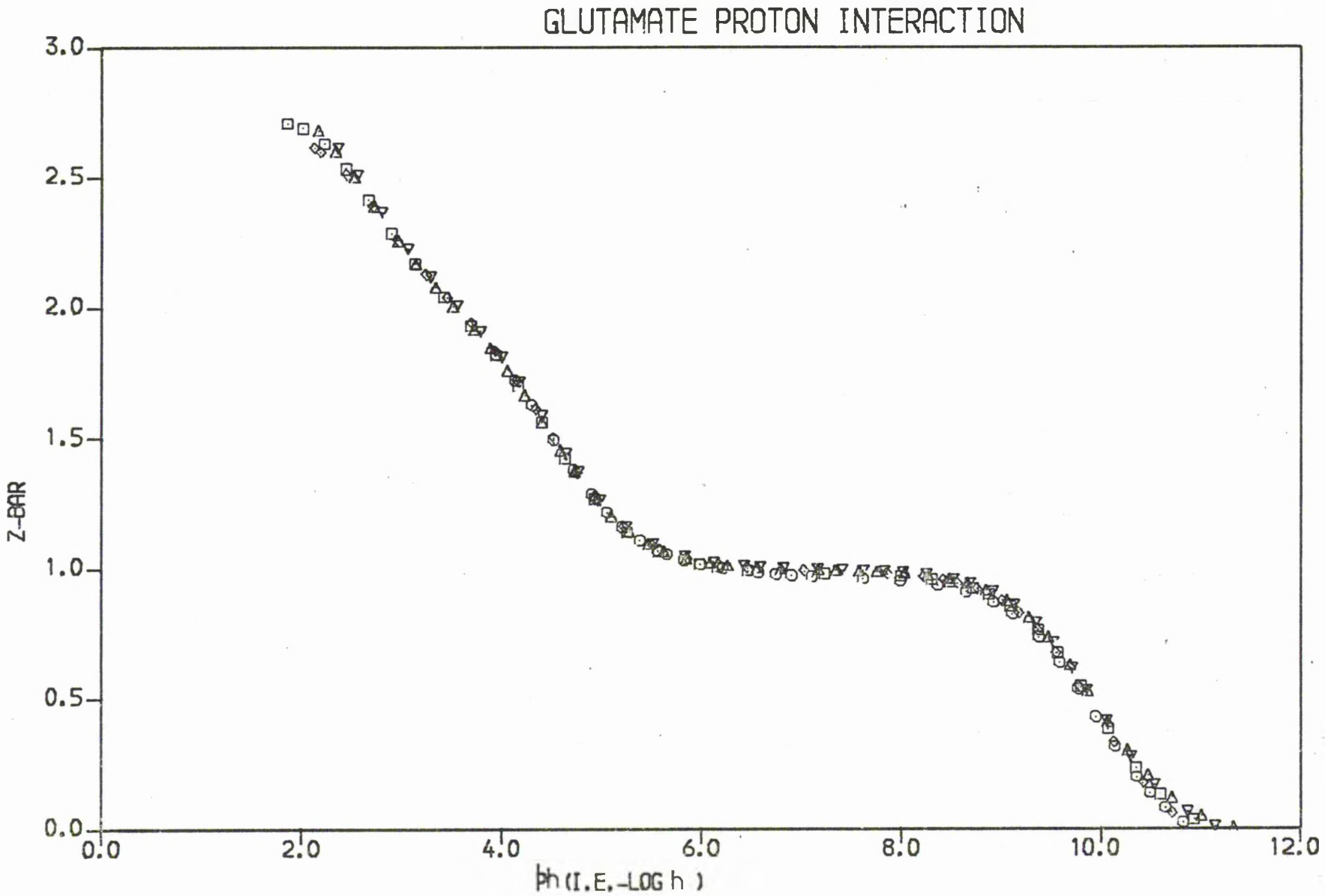


FIGURE 9 : ZPLOT OF DATA FROM APPENDIX 1 - TABLE 1

first of which may be assigned to the protonation of the amino group. However, the other two values cannot be definitely assigned to the protonation of any one site since some concurrent occupation of the two carboxylate sites will occur. Nevertheless, glutamate has donor oxygens separated by five other atoms and it appears that the 4.518 value refers predominantly to a carboxylate group such as occurs in acetate (4.52) [100] (i.e. the γ carboxyl group) and the 2.572 value to a carboxylate group that is α to an amino group such as occurs in glycinate (2.68) [61] or cysteinate (2.44) [101].

The formation constants reported here are generally higher than those of other workers [65,102-104], but this is as expected for our work at higher background ionic strength.

ETHYLENEDIAMINETETRAACETATE

Experimental results are shown in appendix 1 - table 2 and in figure 10.

It was found that if the starting solution was made very acid then some non-superimposability of formation curves occurred. However, this problem was avoided, and high $\bar{Z}$ still attained, by titrating acid into an alkaline ligand solution. This displacement of curves may be due to protonation of the amino nitrogens and the setting up of a non-equilibrium state. The possibility of these nitrogens becoming protonated was discussed as early as 1947 [105] and has been shown to occur more recently [106,107]. Anderegg [108], in fact, has found all six groups to be protonated at low pH.

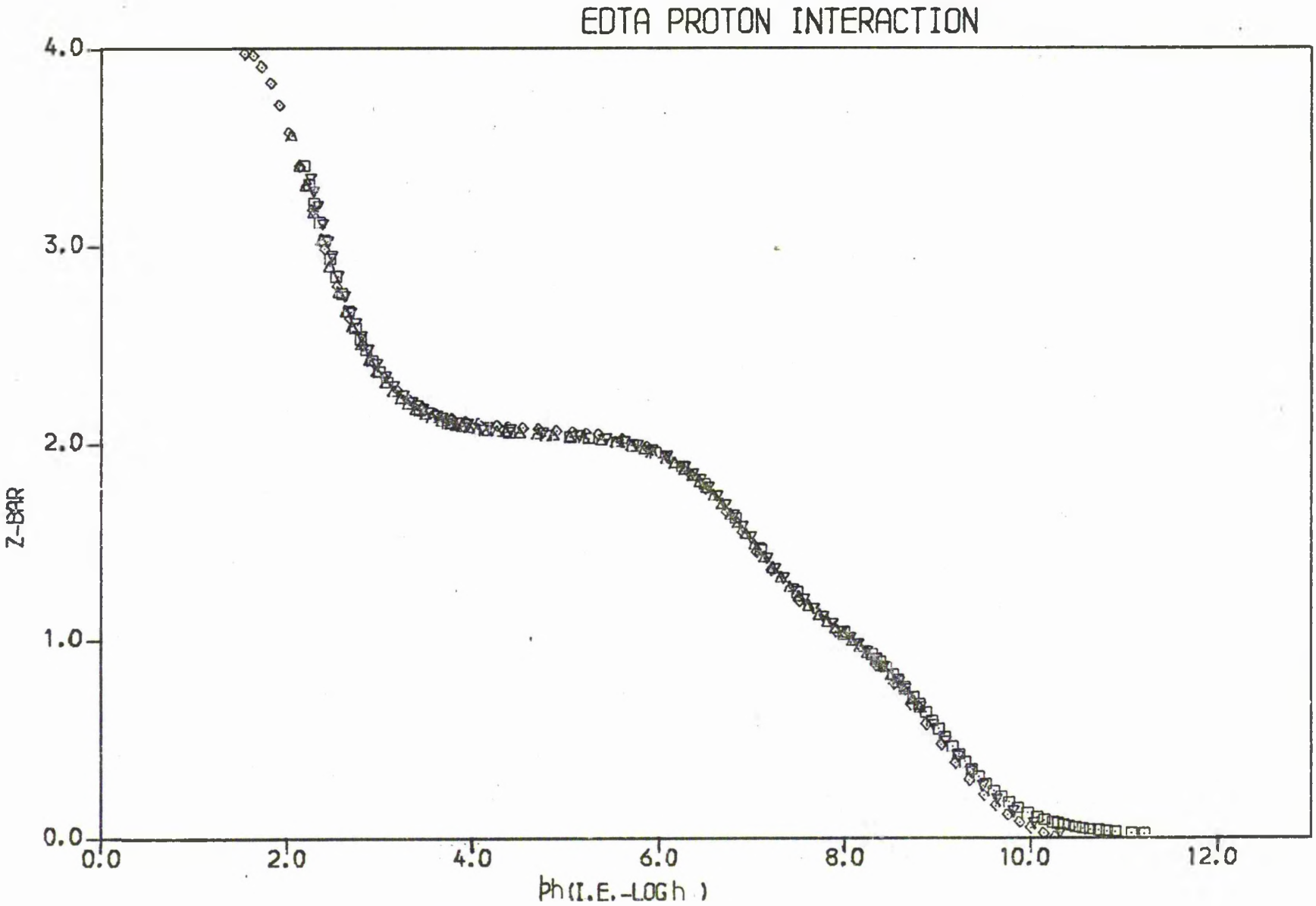


FIGURE 10 : ZPLOT OF DATA FROM APPENDIX 1 - TABLE 2

The computed log K values are 9.060, 7.040, 2.580 and 2.273 which again cannot be assigned to individual sites. These values are in general agreement with those reported in the literature [65].

The sites of protonation of the edta molecule are thought to depend on the extent of protonation. AH_4 may be protonated on two amino and two carboxyl groups [105-107], possibly with some hydrogen bonding to the two free carboxyl groups [109], or on the four carboxyl groups [110]. However, it is generally thought that AH_2 is protonated on two amino groups [106,107,110-113] and AH on one amino group [106,110,112] although in each case hydrogen bonding may be involved [109].

GLUTATHIONATE

Experimental results are shown in appendix 1 - table 3 and in figure 11.

The computed log K values are 9.881, 9.162, 3.819 and 2.595 the first two of which can be assigned to the protonation of the sulphydryl and amino groups and the second two to the carboxyl groups although some concurrent site occupation will occur and so these are composite values.

These constants generally agree with those of other workers when the different conditions are taken into account [65,114].

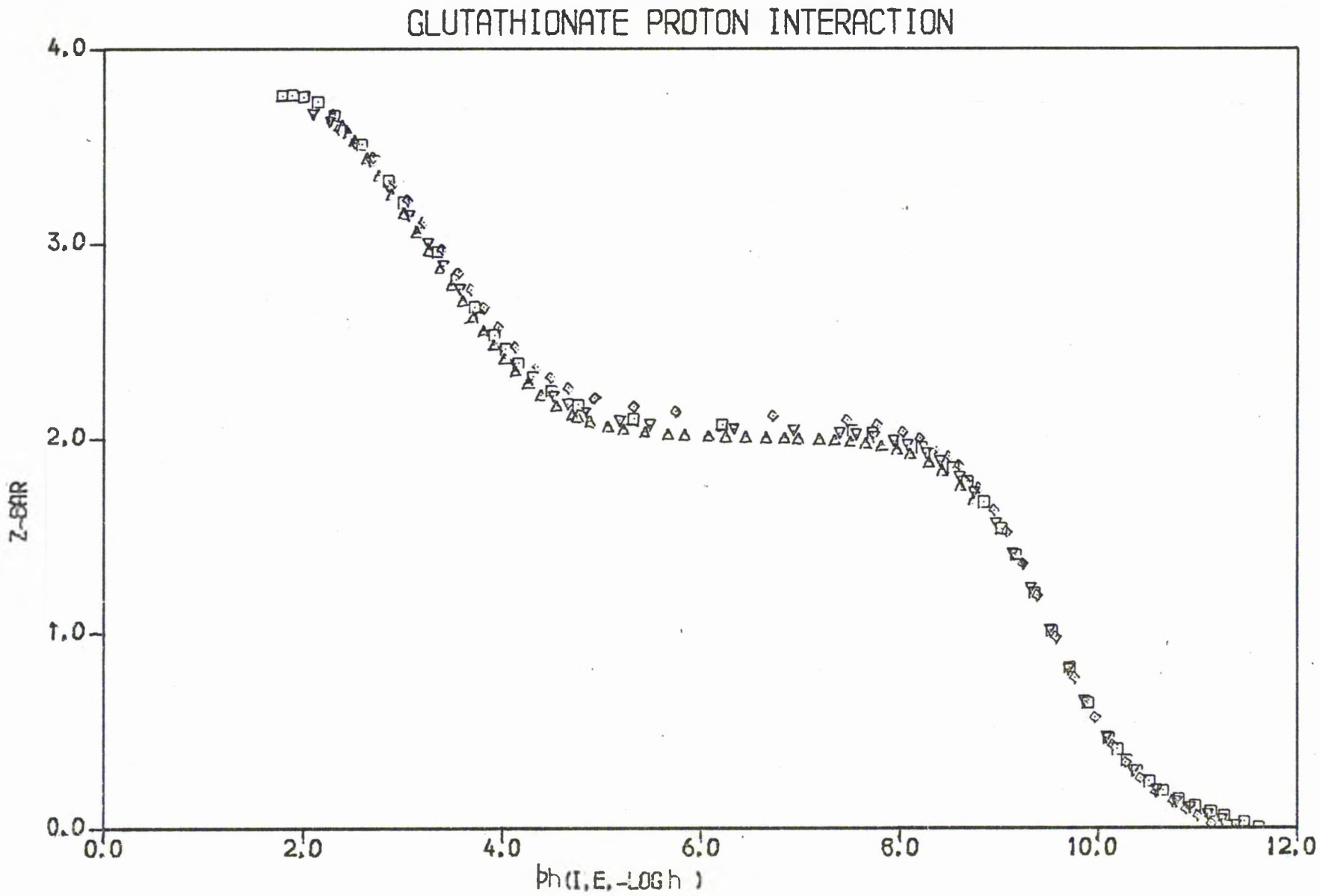


FIGURE 11 : ZPLOT OF DATA FROM APPENDIX 1 - TABLE 3

GLYCINATE

Experimental results are shown in appendix 1 - table 4 and in figure 12.

The computed log K values are 10.070 and 2.682 which may be assigned to the protonation of the amino and the carboxyl groups respectively. These values are higher than those obtained by other workers[65,103,115-121] but this is as we would expect at higher ionic strength.

GLYCYLGLYCINATE

Experimental results are shown in appendix 1 - table 5 and in figure 13.

The computed log K values are 8.562 and 3.510 which can be assigned to the protonation of the amino and carboxyl groups respectively. These values are in agreement with other workers results [65,116,117,122,123] considering the different conditions used.

GLYCYLGLYCYLGLYCINATE

Experimental results are shown in appendix 1 - table 6 and in figure 14.

The computed log K values are 8.601 and 3.634 which again may be assigned to the protonation of the amino and carboxyl groups respectively. These values are in general agreement with those of other workers [65,122,124].

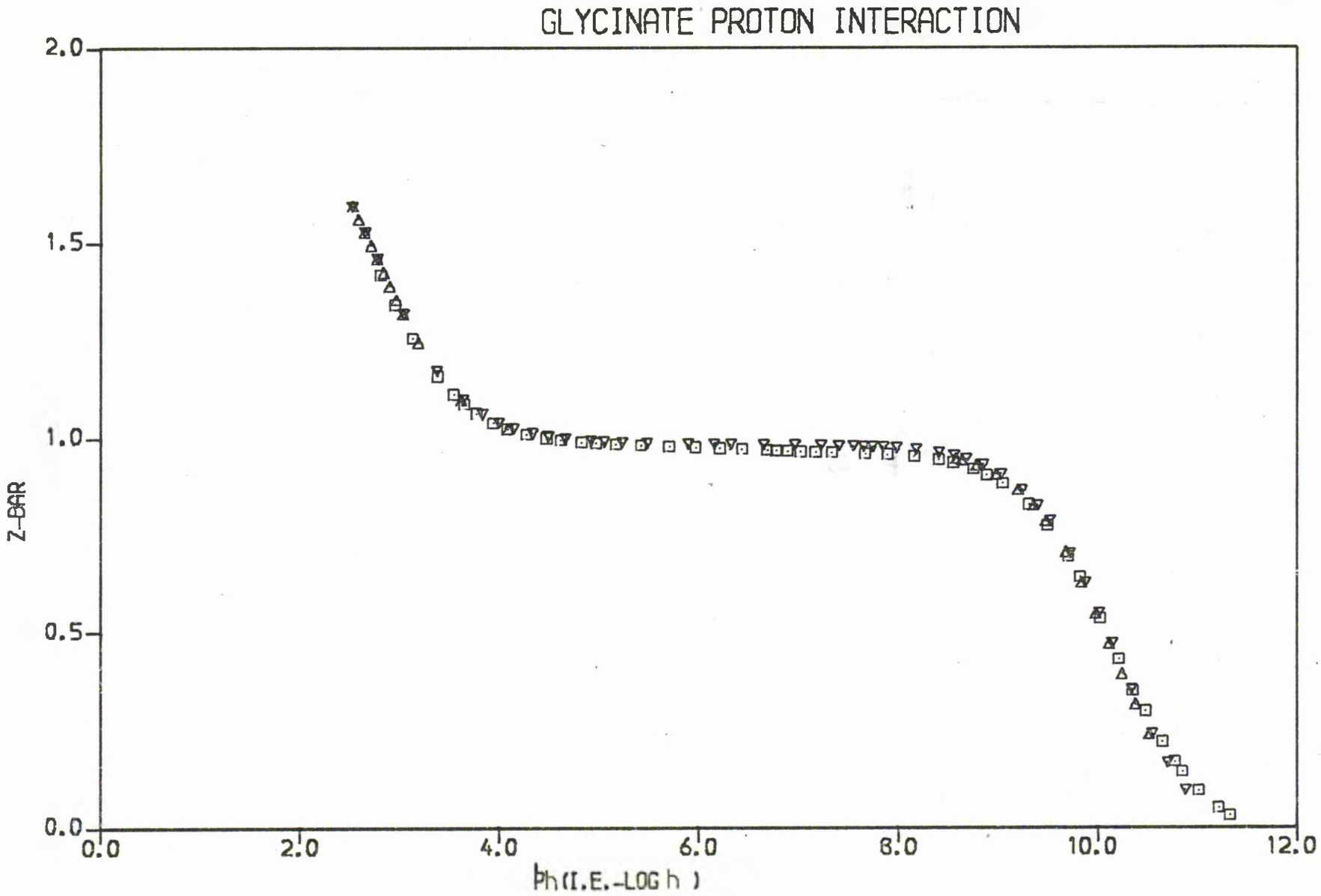


FIGURE 12 : ZPLOT OF DATA FROM APPENDIX 1 - TABLE 4

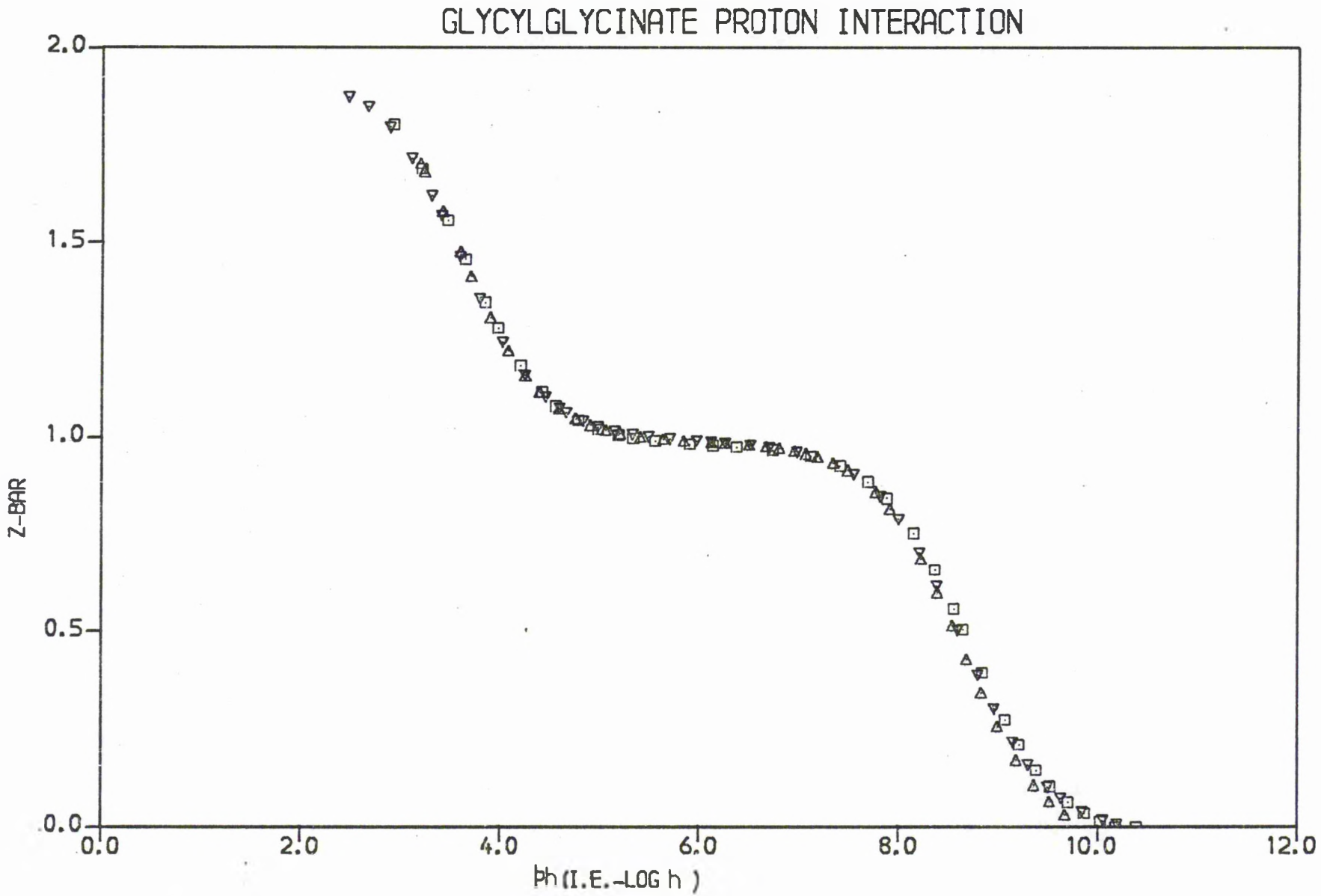


FIGURE 13 : ZPLOT OF DATA FROM APPENDIX 1 - TABLE 5

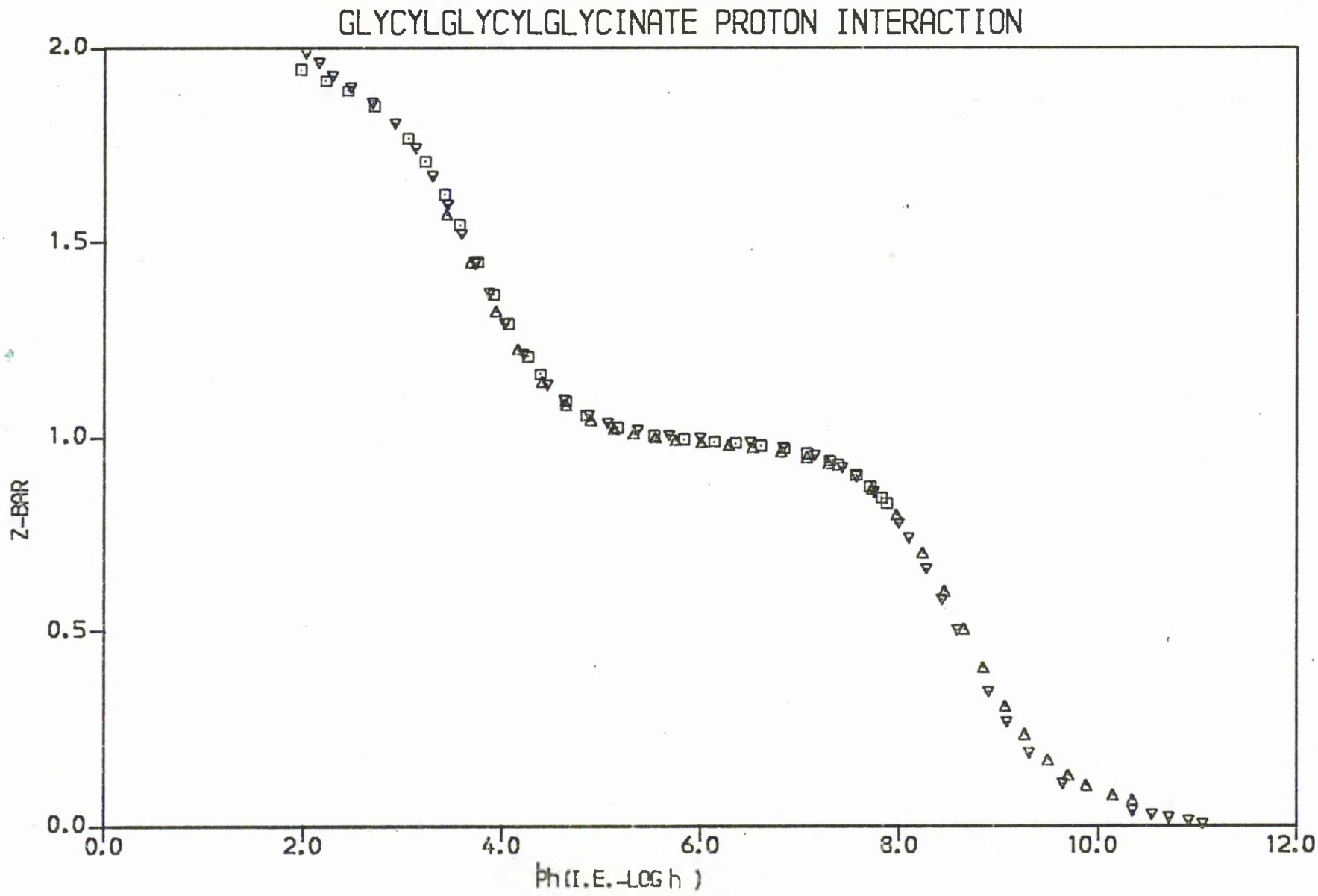


FIGURE 14 : ZPLOT OF DATA FROM APPENDIX 1 - TABLE 6

TABLE 5

LOG FORMATION CONSTANTS FOR LIGAND ANION-PROTON INTERACTIONS AT 25°C, I = 3.00M(Na^+)ClO_4^-

	log β_{pqr}				
	101	102	103	104	n[a]
Glutamate	9.8947 ± 0.0051	14.4130 ± 0.0080	16.9854 ± 0.0190		183
Ethylenediaminetetraacetate	9.0599 ± 0.0049	16.1001 ± 0.0073	18.680 ± 0.017	20.953 ± 0.014	263
Glutathionate	9.881 ± 0.020	19.043 ± 0.018	22.861 ± 0.019	25.456 ± 0.020	182
Glycinate	10.0702 ± 0.0072	12.752 ± 0.012			119
Glycylglycinate	8.5621 ± 0.0072	12.0716 ± 0.0091			115
Glycylglycylglycinate	8.6006 ± 0.0085	12.234 ± 0.012			101

[a] n = number of experimental observations

Metal-Ligand Complex Formation Constants

A solution of metal, ligand and acid is titrated with a solution of alkali. For each metal-ligand system several experiments were performed using different ratios of ligand: metal in order to identify hydroxy, protonated or polynuclear species. In all cases non-superimposable formation curves were obtained indicating the presence of one or more of these non-simple complexes.

All experimental data for the metal-ligand complex formation work is given in appendix 2 and the determined constants, with their standard deviations and the number of experimental observations in table 7. In all cases the four lead-hydroxy complexes found by Olin [36] were included in the calculations.

LEAD(II)-ASPARTATE

Experimental results are shown in appendix 2 - table 1 and in figure 15.

The maxima seen in the formation curves at high pa are characteristic of the presence of protonated species. The complexes, pqr, which were searched for in the computer analysis were those with p1,2; q1 and r-1 to 4 and those which gave convergence were 110, 210, 111, 112, 211 and 212 with log β values of 6.878, 10.014, 12.695, 16.167, 19.181 and 24.985 respectively. It was found impossible to refine all of these constants together in SCOGS because of exponent overflow and so the results were determined from MINIQUAD only.

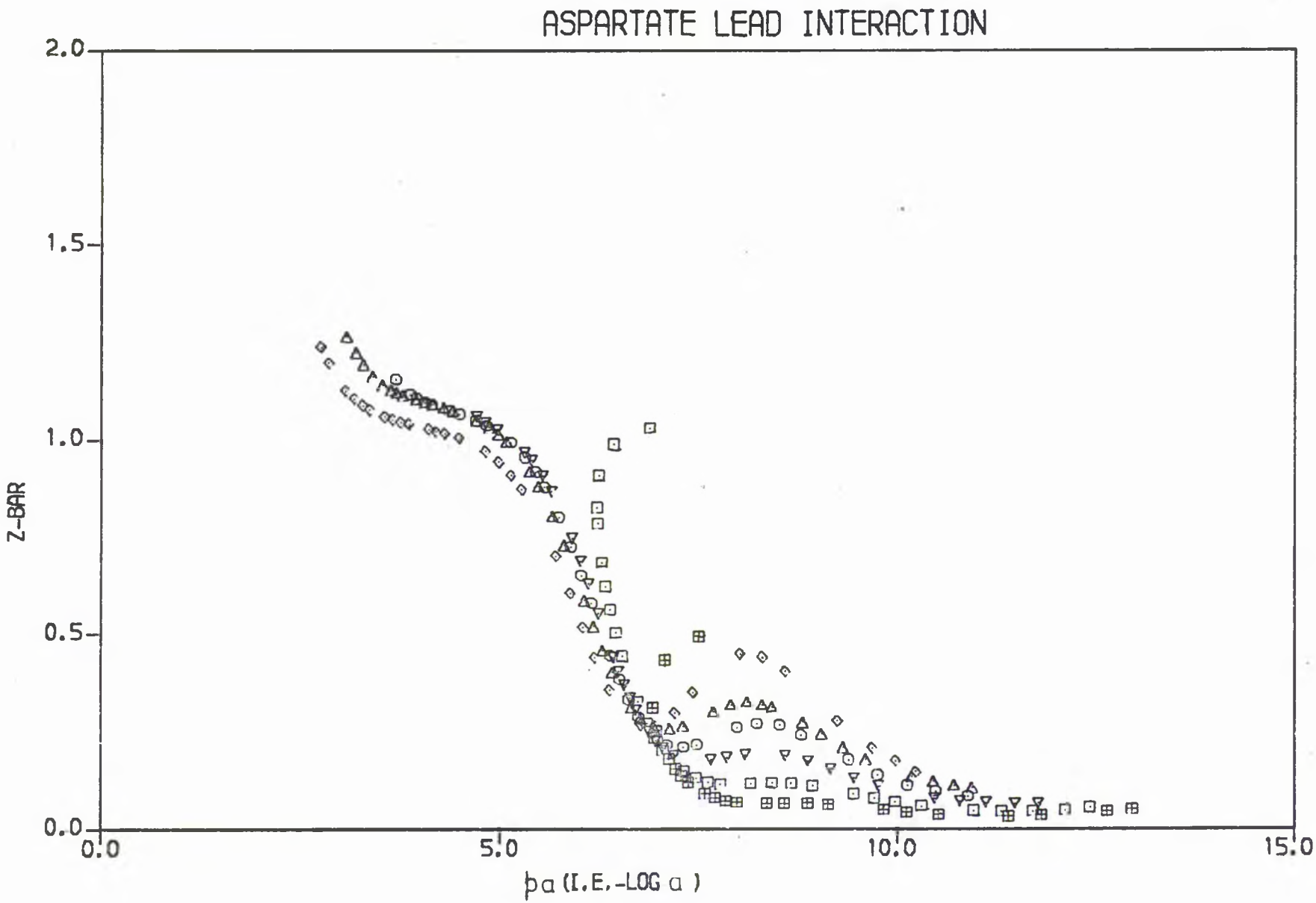


FIGURE 15 : ZPLOT OF DATA FROM APPENDIX 2 - TABLE 1

A simulated set of formation curves obtained from PSEUDOPLOT using the above constants and the aspartate protonation constants from reference 101 is shown in figure 16. As can be seen, good agreement is found between the experimental and simulated curves.

Other workers have reported log formation constants only for the species 110 and 210 with values of 5.88 and 7.38 [125] and 6.02 and 8.18 [126] respectively. These constants are considerably lower than those reported here which may be partly explained by the difference in conditions used. However, a COMPLOT model of the system (figure 17) shows that at no pH are the 110 and 210 species present alone and failure to consider the other species present will effect the constants calculated.

The relatively high log formation constant found for the 110 complex compared to other amino acids (e.g. asparaginate = 4.902 and phenylalaninate = 4.314 from table 4) suggests that aspartate is bound tridentately to lead(II) in this species. However, we cannot reach any definite conclusions regarding structures from formation constant data alone.

LEAD(II)-CYSTEINATE

Experimental results are shown in appendix 2 - table 2 and in figure 18.

With this ligand it was possible to work at only low concentrations of lead(II) and at ligand to metal ratios of more than 2:1 because of precipitation. A portion of the precipitate was collected and its analysis was in close agreement with that of the 110 species which is uncharged and so might be expected not to be very soluble.

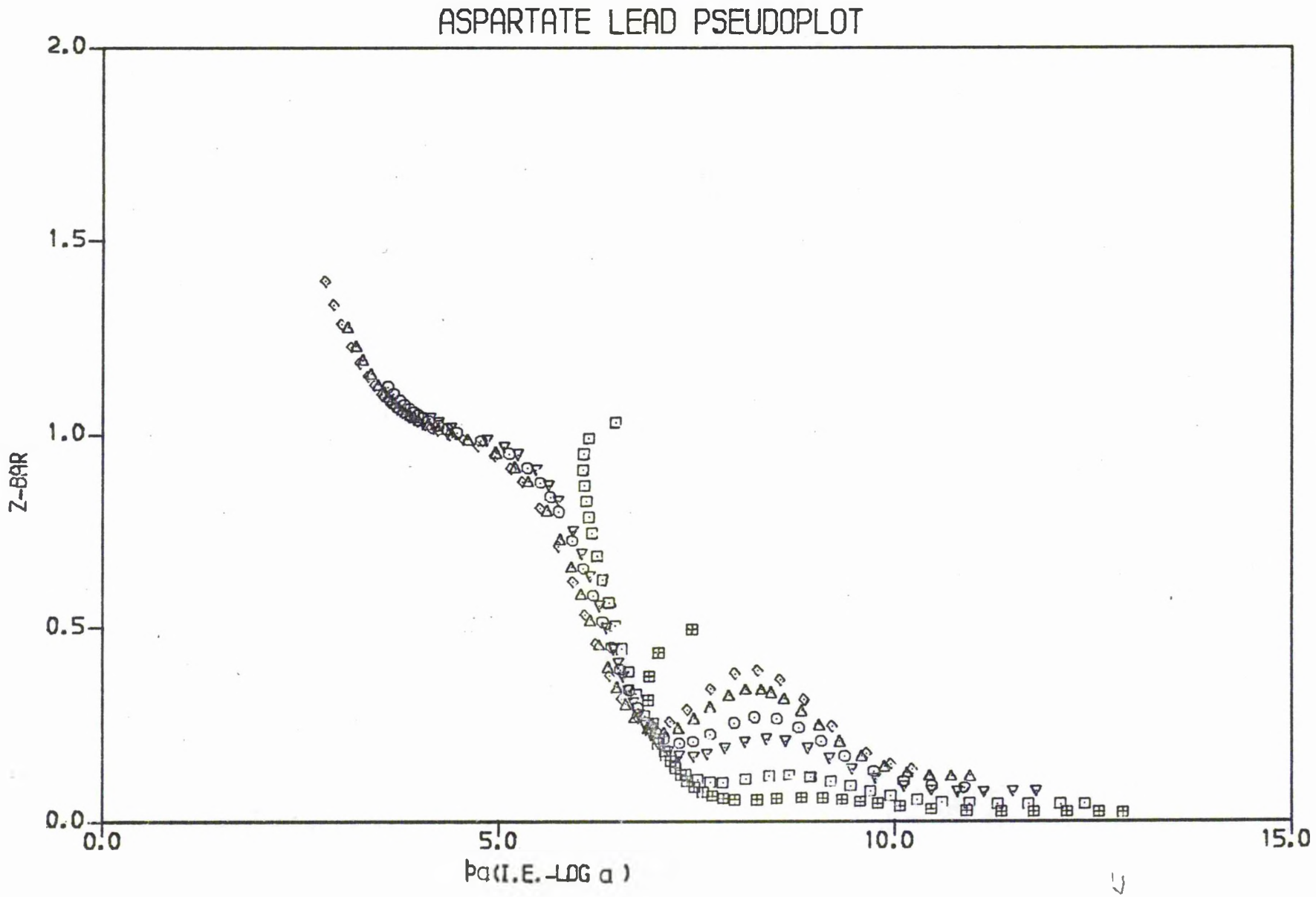


FIGURE 16 : PSEUDOPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

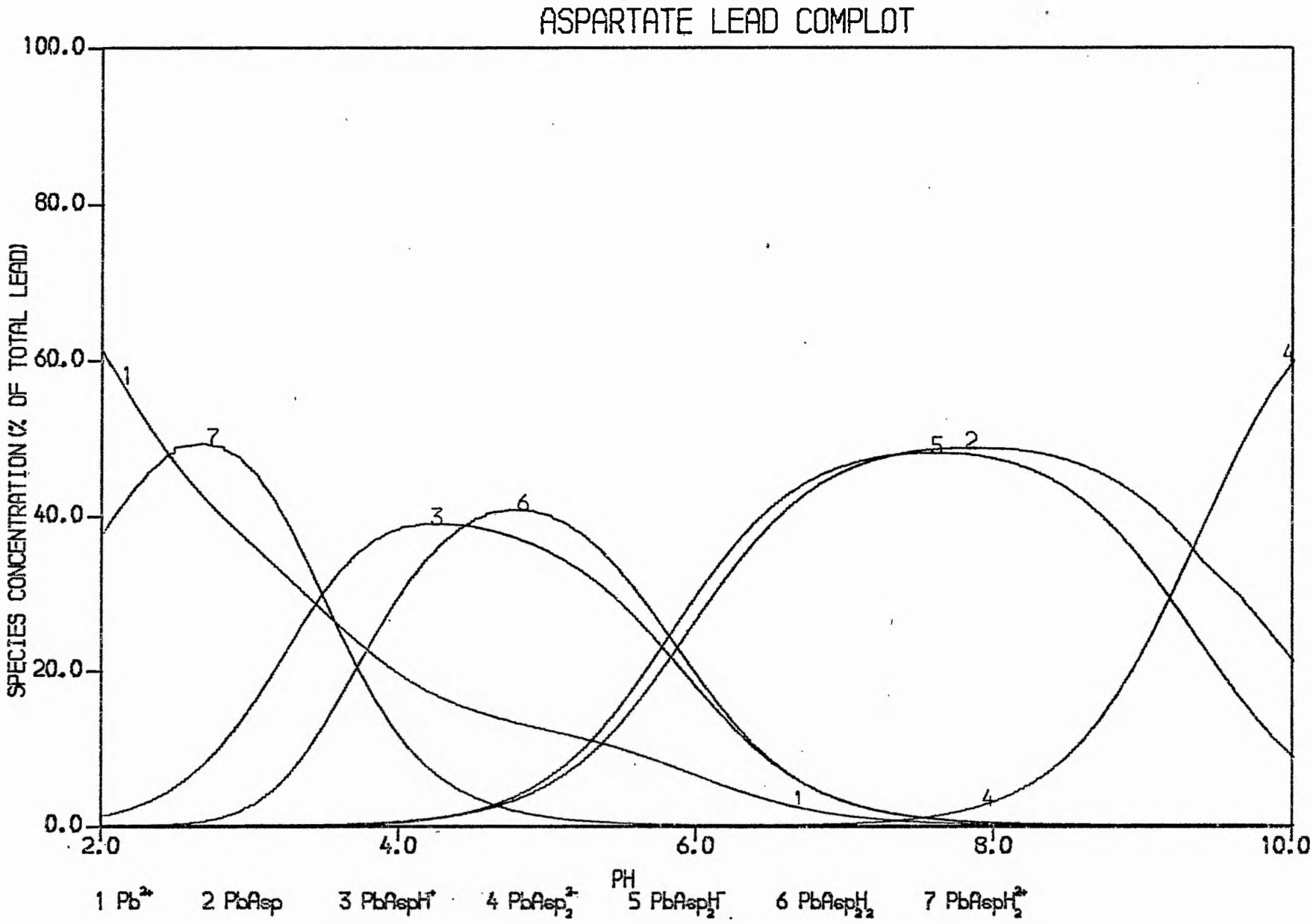


FIGURE 17 : COMPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

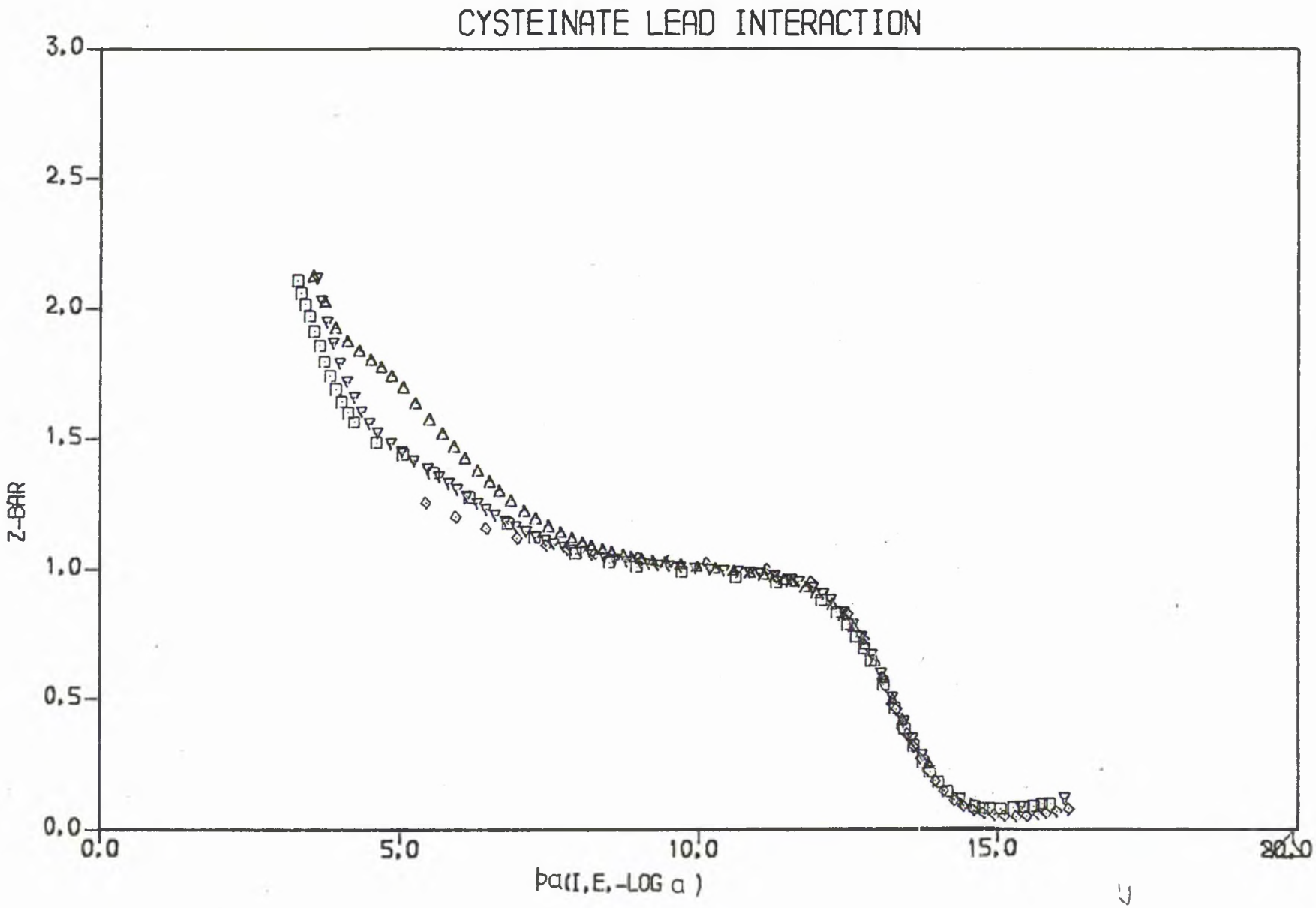


FIGURE 18 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 2

The complexes searched for were those with p 1 to 3; q 1, 2 and r -2 to 2. Those which gave convergence in MINIQUAD were 110, 210, 111, 211 and 21-1 with log β values of 13.213, 18.571, 17.347, 27.476 and 7.33 respectively. The last of these, however, has a high standard deviation, only marginally improves the sum of squared residuals and does not improve the PSEUDOPLOT fit. A COMPLOT model of the system (figure 19) shows that this species would be of only minor importance and then only at high pH. Thus its actual presence in the system is very doubtful.

A simulated set of formation curves obtained from PSEUDOPLOT using the formation constants from table 7 and the cysteinate protonation constants from reference 101 is shown in figure 20.

Other workers have reported formation constants for the 110, 210 and 310 species [127-130] but there are no reports of the protonated species. Literature values of log β_{110} range from 11.39 to 12.75 and of log β_{210} from 16.60 to 16.91. These constants have all been measured at $I \leq 0.15$M and so are lower than those reported here.

Proposed structures for these complexes will be discussed in Chapter 8.

ZINC(II)-CYSTEINATE

Experimental results are shown in appendix 2 - table 3 and in figure 21.

By comparison with figure 18, we see that these formation curves are a completely different shape to those for the lead(II)-cysteinate

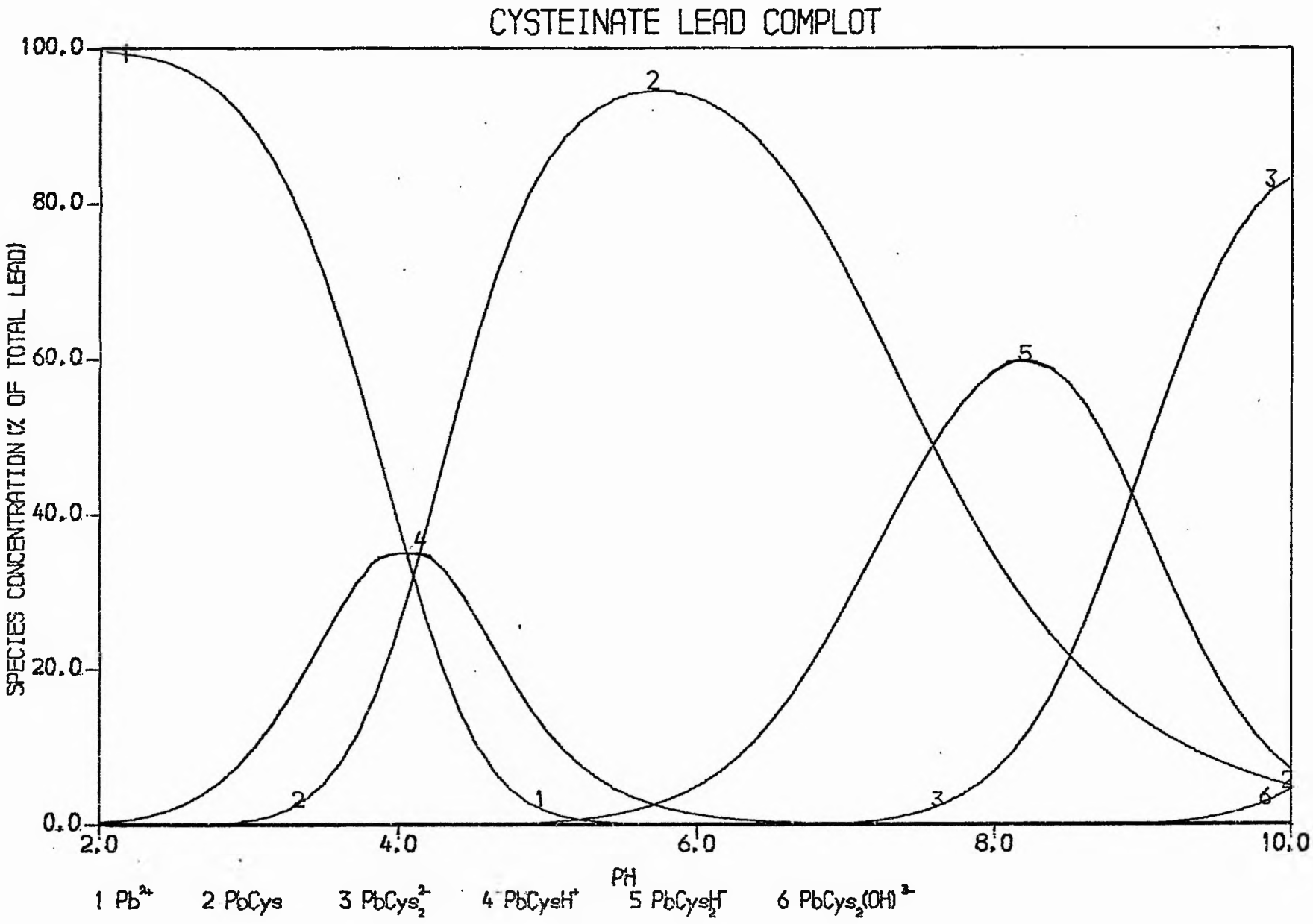


FIGURE 19 : COMPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7 AND LOG β_{21-1} = 7.33

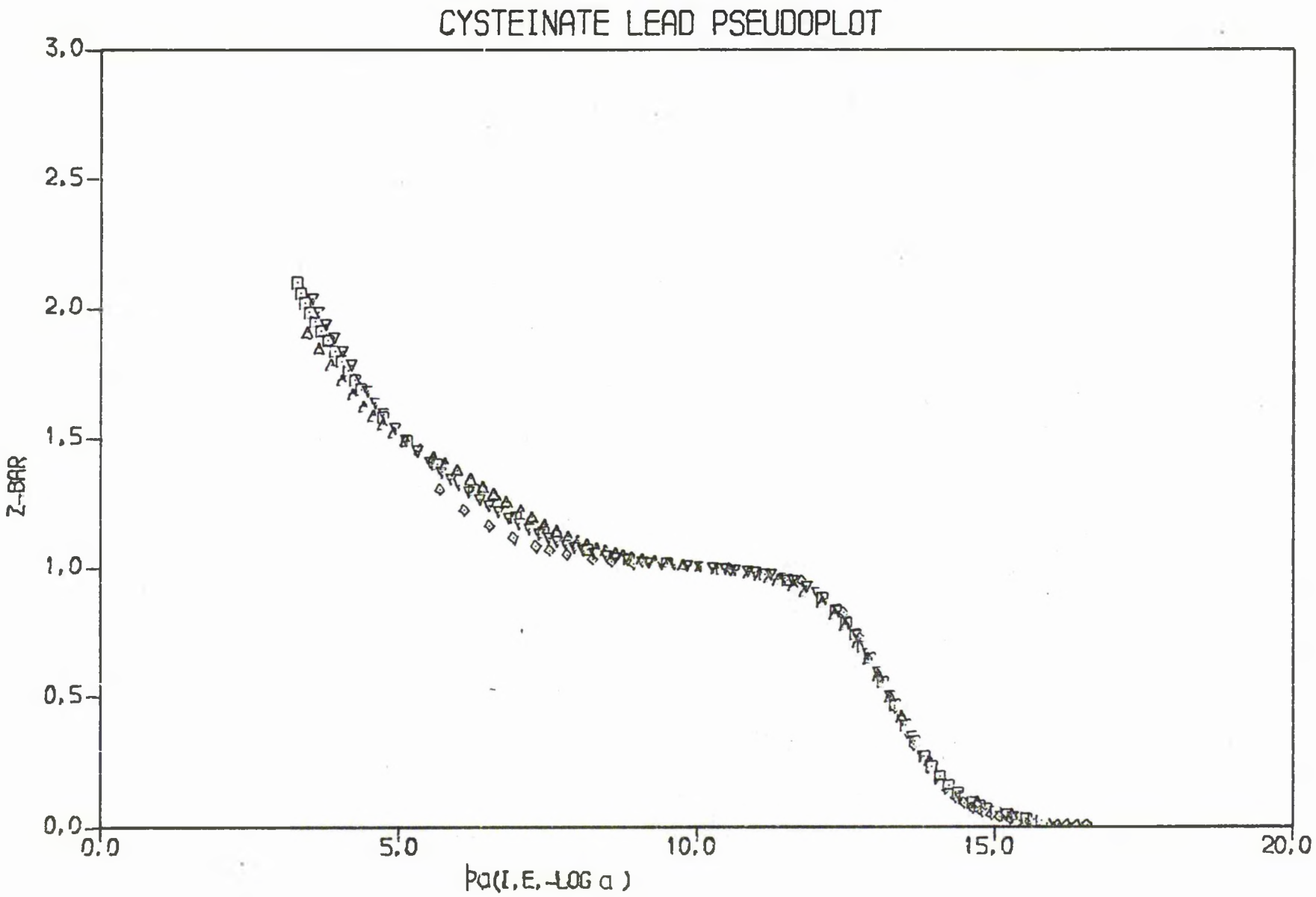


FIGURE 20 : PSEUDOPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

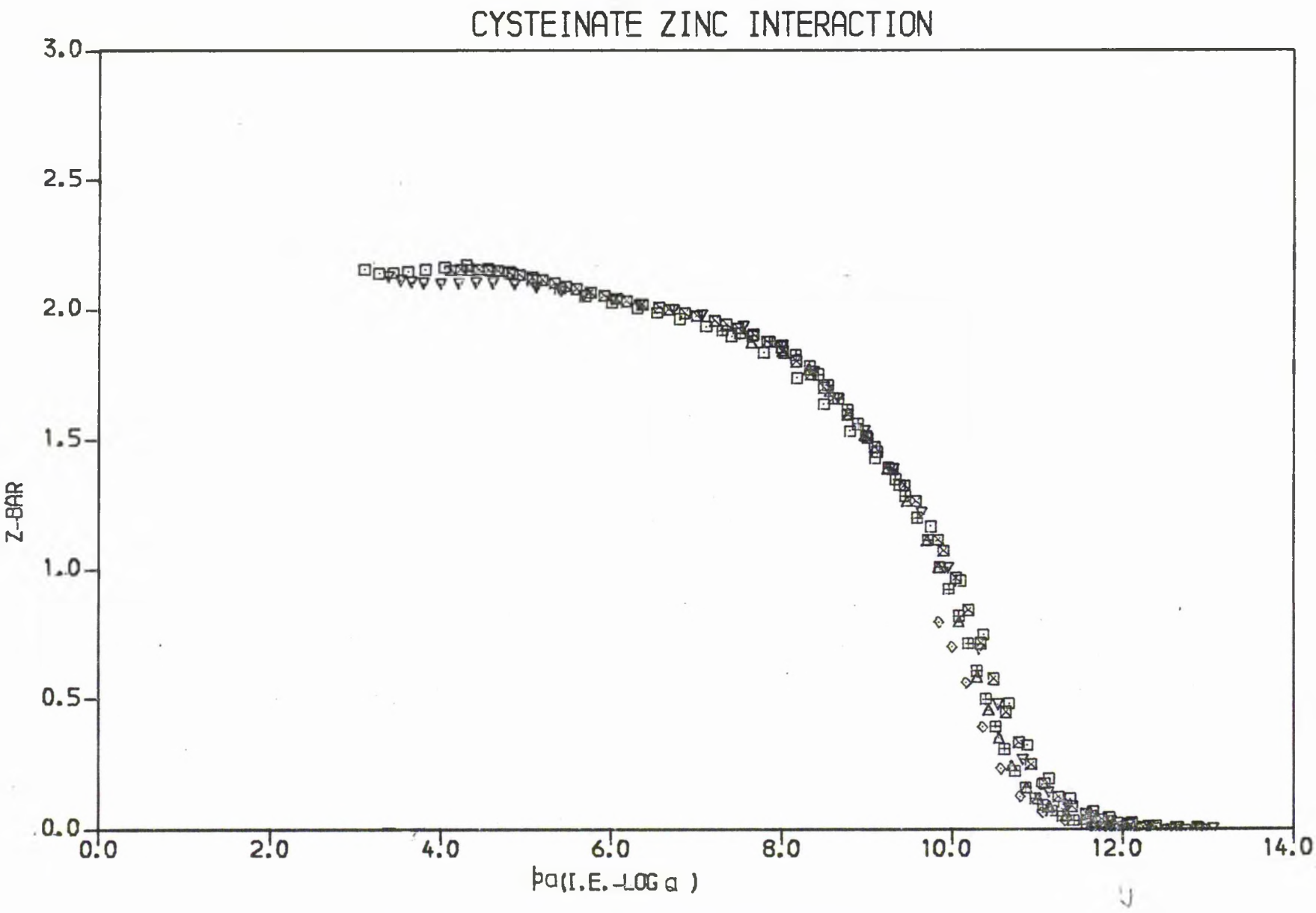


FIGURE 21 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 3

interaction. In figure 21 the curves show no tendency to level off at $\bar{Z}$ = 1.0 indicating that the 110 complex is not very stable with respect to the 210. Also there is some crossing over of the formation curves which suggests that some polynuclear species are present.

The complexes searched for were those with p 0 to 4; q 1 to 3 and r -1 to 2 and those giving convergence were 210, 211, 212, 430 and 431 with log β values of 19.395, 25.856, 31.879, 46.247 and 52.50 respectively.

A simulated set of formation curves obtained from PSEUDOPLOT using these constants is shown in figure 22.

The fact that no 110 complex is found means that log K_1 < log K_2 and so complexation does not occur stepwise but simultaneously. Similar behaviour has been found previously for nickel(II) and zinc(II) [129,131] and the explanation is thought to lie in the ability of sulphur to accept electrons from the metal ion by $\bar{\pi}$ bonding [132] thus facilitating the addition of a second sulphur atom to the metal.

Several workers have determined formation constants for the 110, 210 and 310 complexes [65,129,130, 133]. However, only Perrin and Sayce[131] have found evidence of protonated and polynuclear species. The formation constants reported here agree well with theirs considering that their working conditions were 20°C, I = 0.1M $NaClO_4$.

Since no 110 complex is found, it is likely that cysteine acts as a bidentate ligand to zinc(II) to give a stable tetrahedral structure for the 210 species. The high value of the formation constant for this species suggests that it is the amino and sulphydryl groups which are involved in the bonding so leaving the carboxyl group free to be protonated. Bidentate binding of cysteinate to zinc(II) has been

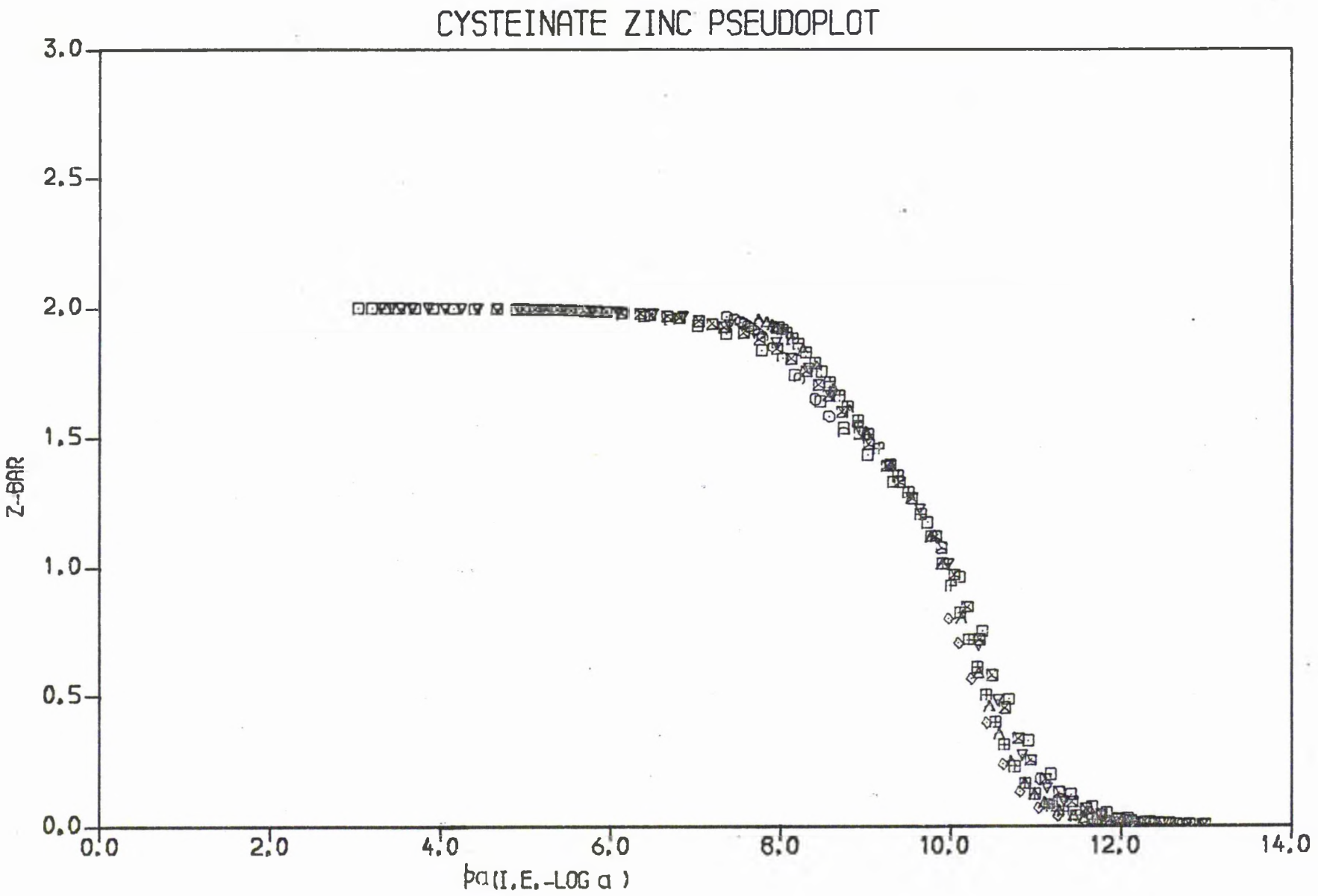


FIGURE 22 : PSEUDOPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

postulated by several other workers [127-129,133-135].

LEAD(II)-GLUTAMATE

Experimental results are shown in appendix 2 - table 4 and in figure 23.

Again we see evidence of a $\bar{Z}$ maximum at high pa suggesting the presence of protonated species although this is not as marked as in the lead(II)-aspartate case. The complexes which were searched for were those with p 1,2; q 1 and r -1 to 2 those giving convergence being 110 and 111 with log β values of 5.344 and 12.173 respectively.

A simulated set of formation curves obtained from PSEUDOPLOT using these formation constants is shown in figure 24 and fits the experimental data well.

Other workers have reported formation constants for the 110 and 210 species [65,136] and again the values are generally lower than those reported here. The formation constant for the 110 species is nearer the expected value for a bidentate amino acid than in the lead(II)-aspartate case and so this ligand may only be bidentate to lead(II).

LEAD(II)-ETHYLENEDIAMINETETRAACETATE

Experimental results are shown in appendix 2 - table 5 and in figure 25. In this case a solution of metal, ligand and alkali was titrated with acid.

The formation curves form a complicated pattern indicating the

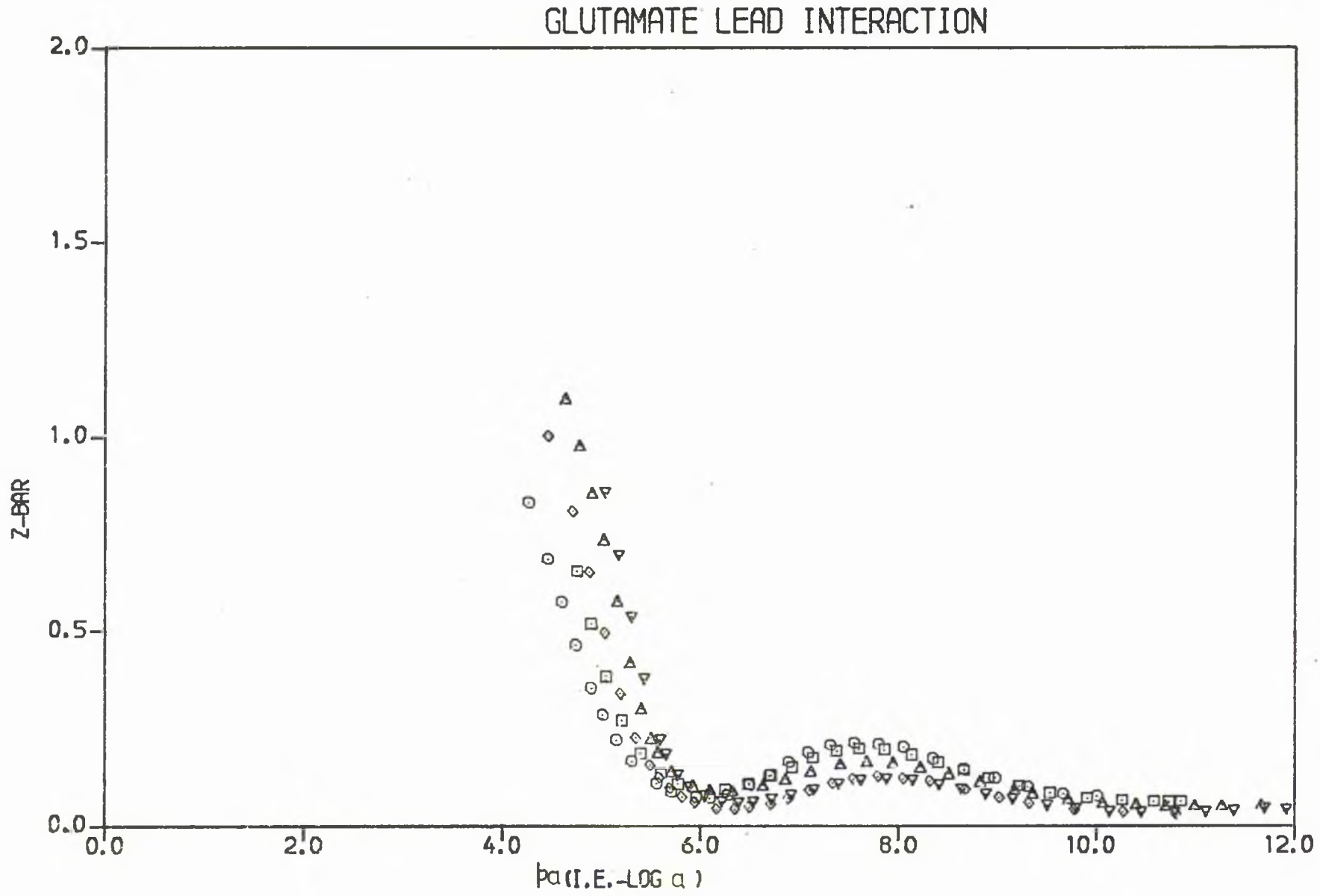


FIGURE 23 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 4

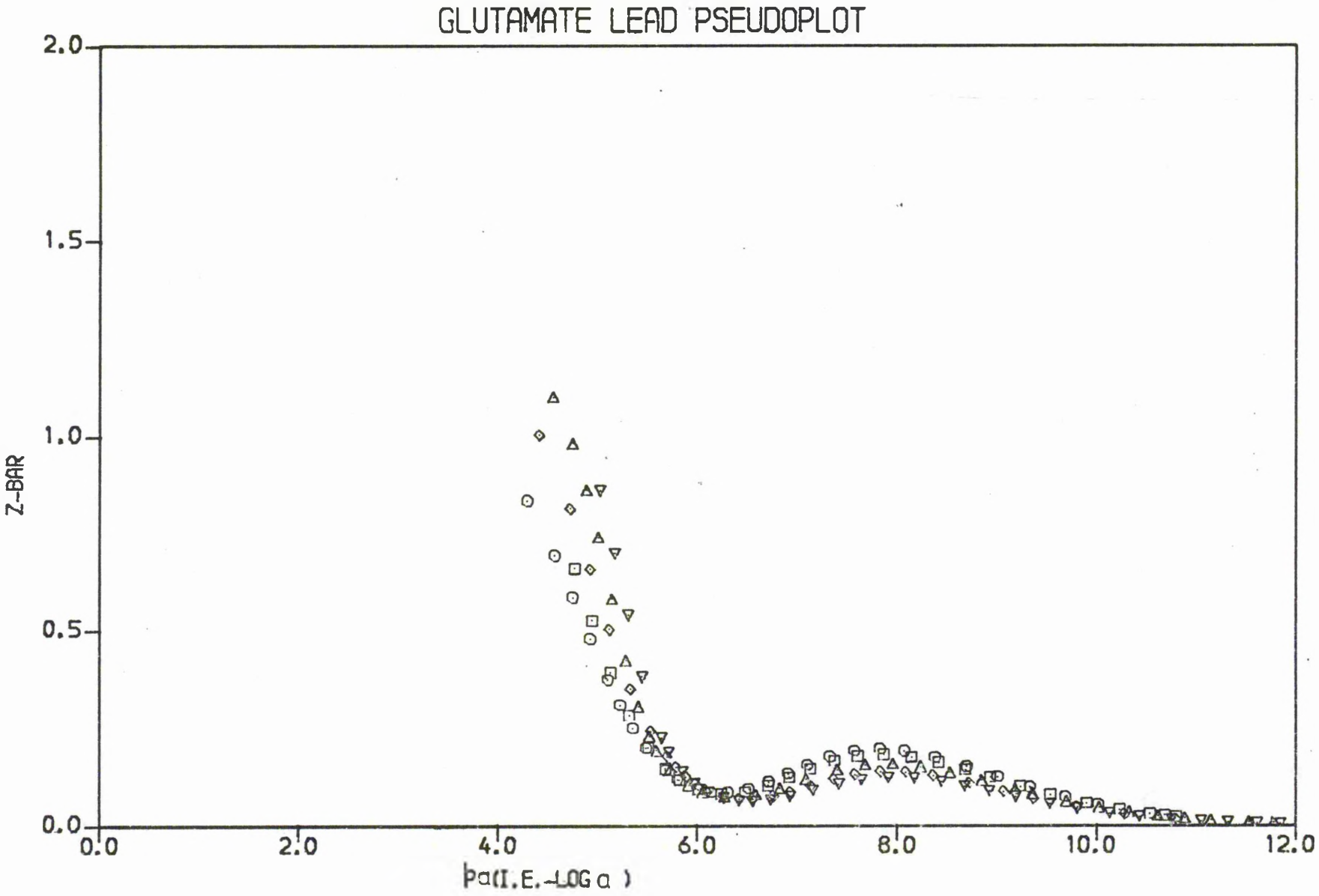


FIGURE 24 : PSEUDOPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

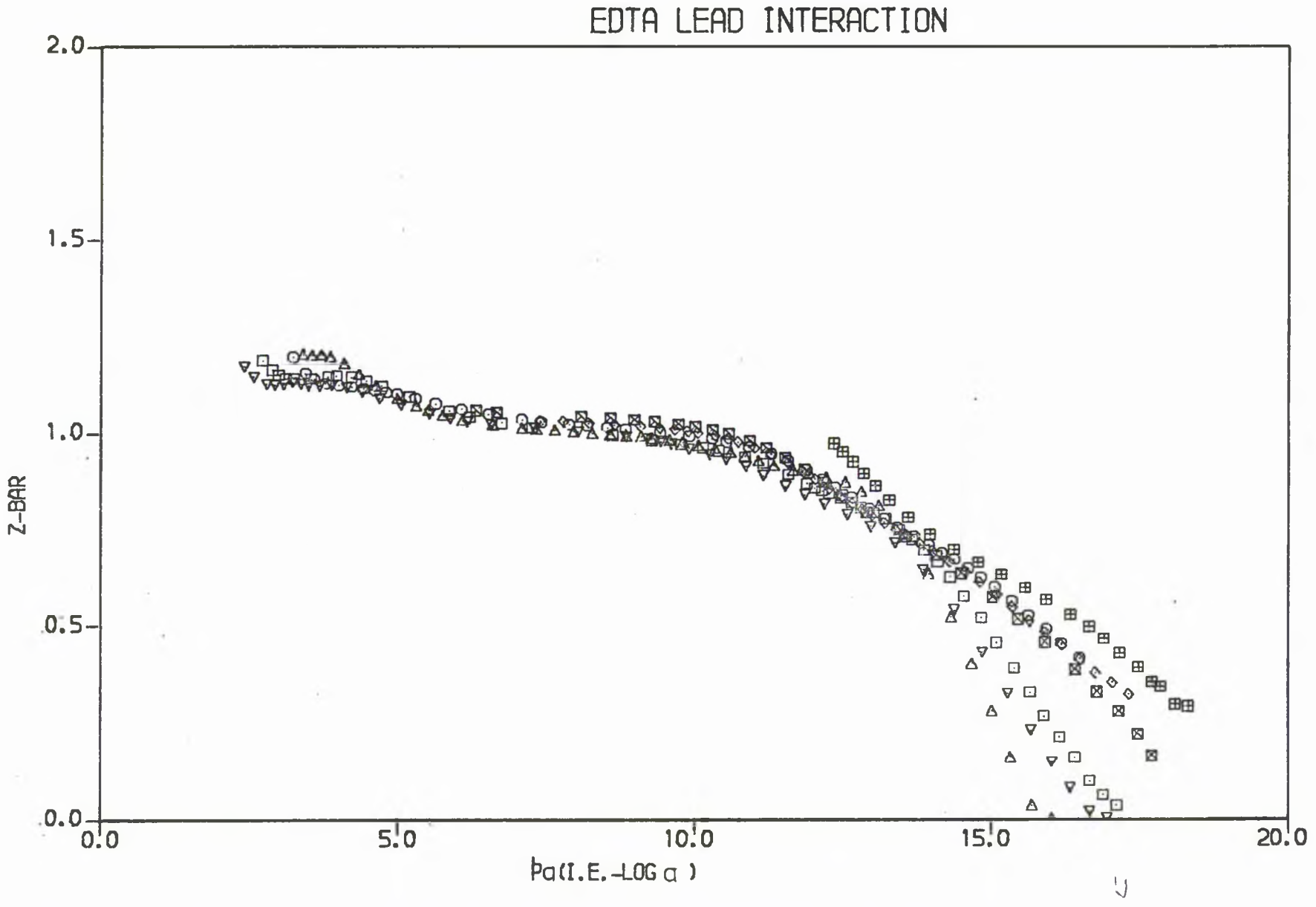


FIGURE 25 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 5

presence of non-simple complexes. The complexes searched for were those with p 1 to 4; q 1 to 4 and r -1 to 4. The 'best' PSEUDOPLOT fit is obtained with 110 and 111 which converge to log β values of 15.186 and 18.010 respectively. As can be seen from figure 26, the simulated curves using these formation constants fit the experimental data very badly. Convergence in MINIQUAD also occurred with species of the type 120, 230 *etc* but these did not significantly improve either the sum of squared residuals or the PSEUDOPLOT fit. Obviously some other species must be present but these have not been determined.

The 111 complex has been detected by other workers [137] as have hydroxy complexes [138]. Literature values [65] for the formation constant of the 110 species are generally higher than those reported here. A similar effect has been found by Sanderson [91] for cadmium(II)-edta complex formation constants determined at 25°C, I = 3.00M(Na^+)ClO_4^-.

ZINC(II)-ETHYLENEDIAMINETETRAACETATE

Experimental results are shown in appendix 2 - table 6 and in figure 27. In this case a solution of metal, ligand and alkali was titrated with acid.

The formation curves show a striking similarity to those found for the lead(II)-edta system (figure 25) and the complexes searched for were those with p 1 to 5; q 1 to 5 and r -1 to 4. Again species of the type x(x+1)o were found to give convergence but with no improvement in the PSEUDOPLOT fit. The 'best' fit was found with 110 and 111 having log β values of 14.873 and 17.965 respectively. A simulated set of formation curves using these constants is shown in figure 28 but they do not satisfactorily fit the experimental data.

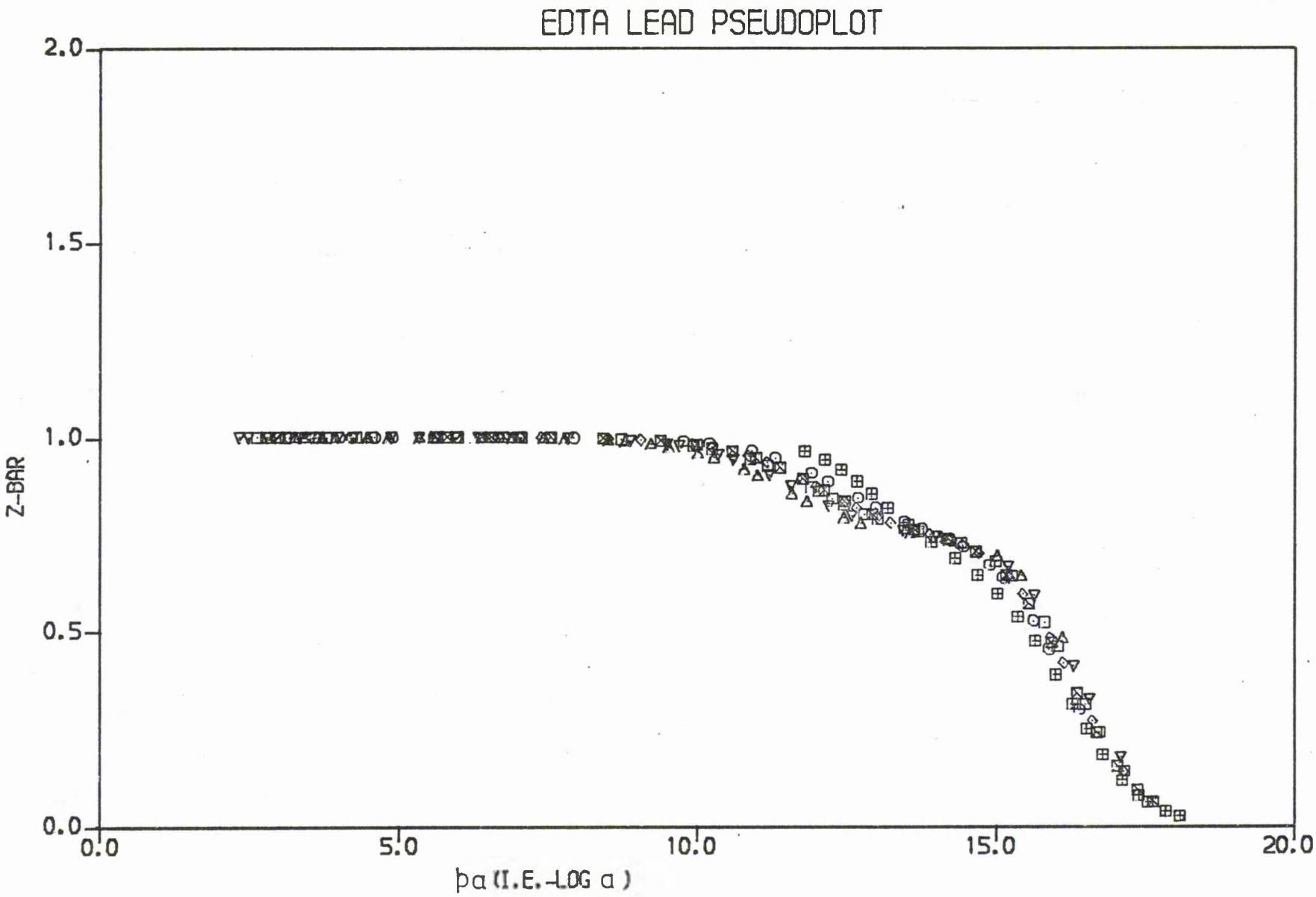


FIGURE 26 : PSEUDOPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

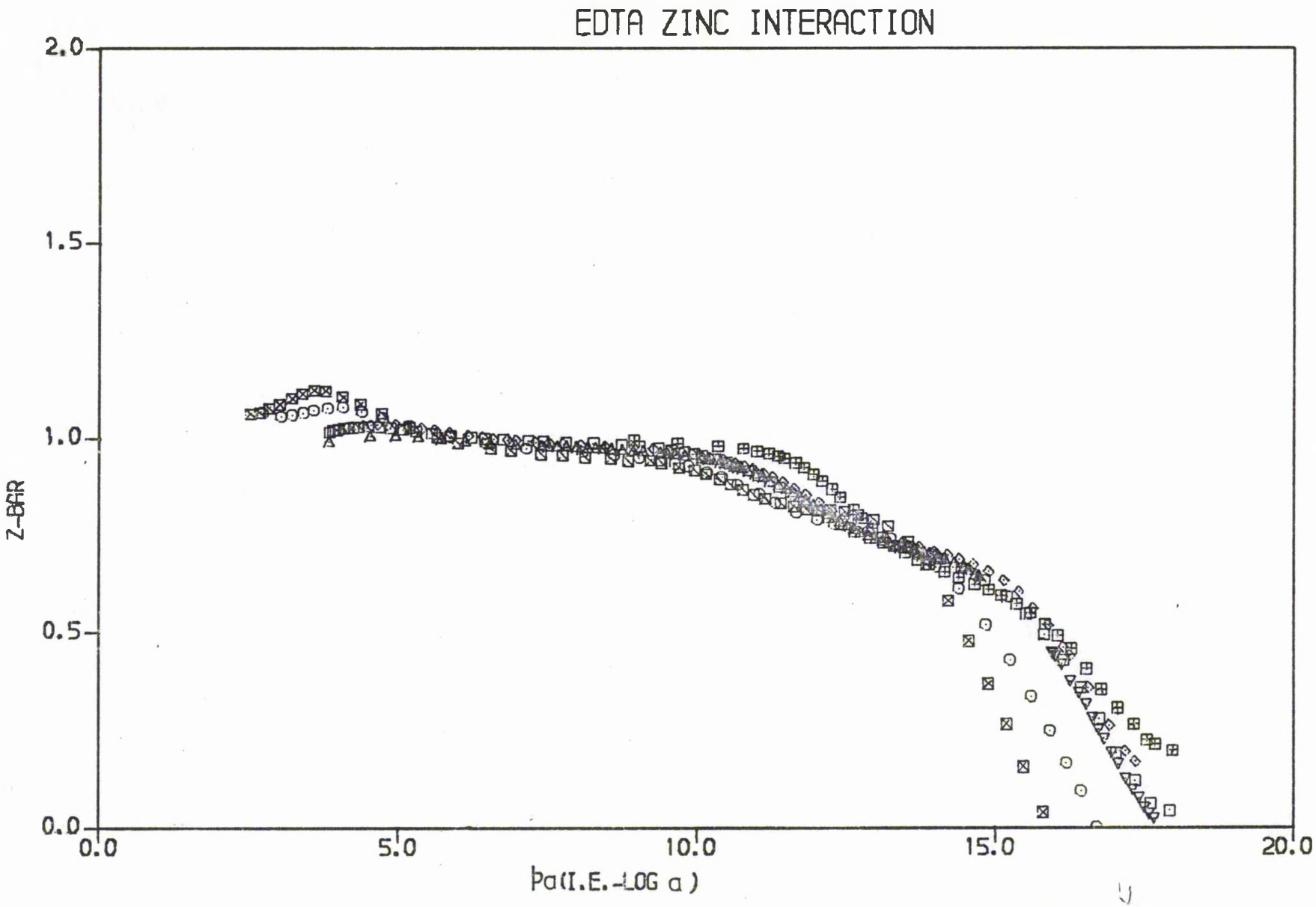


FIGURE 27 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 6

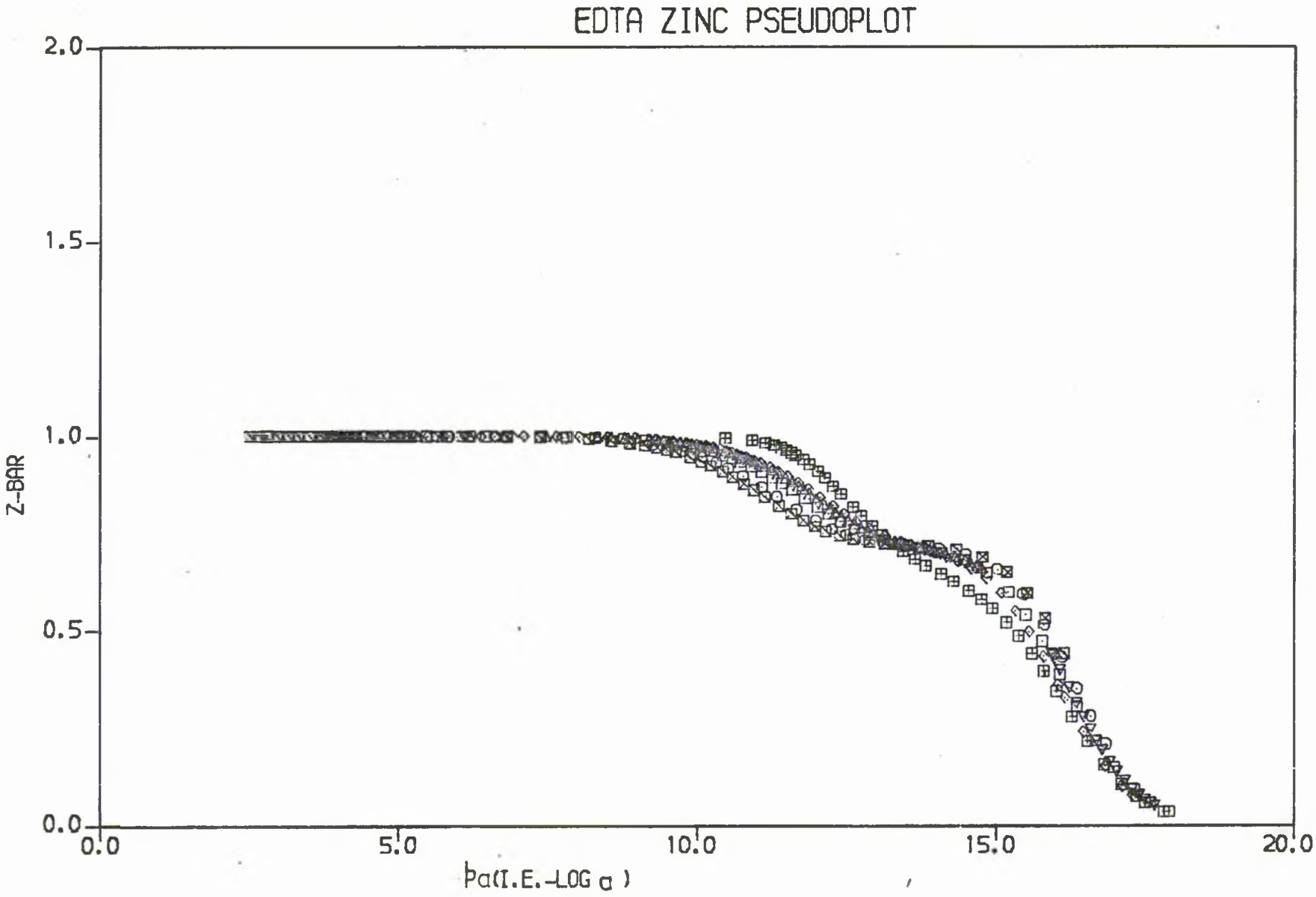


FIGURE 28 : PSEUDOPLOT USING FORMATION CONSTANTS SHOWN IN TABLE 7

The 111 complex [139] and 11-1 complex [138] have been reported by other workers. Again published formation constants [65], generally determined at lower ionic strength, are higher than those reported here.

LEAD(II)-D-PENICILLAMINATE

Experimental results are shown in appendix 2 - table 7 and in figure 29. The D-penicillaminate protonation constants used were from reference 30.

The pattern of formation curves found is reminiscent of that for the lead(II)-cysteinate system showing superimposability at $\bar{Z} < 1$ but divergence at higher $\bar{Z}$.

The species searched for were those with p 1,2; q 1,2 and r -1 to 2, and those giving convergence were 110, 210, 111, 211, 212 and 21-1 with log β values of 14.321, 19.049, 17.723, 27.978, 34.0 and 7.551 respectively. The log β_{212} has a high standard deviation and its inclusion does not improve either the sum of squared residuals or the PSEUDOPLOT fit. A COMPLOT model of the system (figure 30) obtained using the above constants shows that the complexes 212 and 21-1 would be present only in very small amounts and so the values determined for their formation constants are unreliable.

A simulated set of formation curves obtained from PSEUDOPLOT using the constants from table 7 is shown in figure 31.

Formation constants, which are lower than those determined from this work, have been reported [65,129] for the 110, 210 and 310 complexes but not for any of the protonated species.

As is the case for the lead(II)-cysteinate system, log K_1 >> log K_2 suggesting that the carboxylate group is involved in the

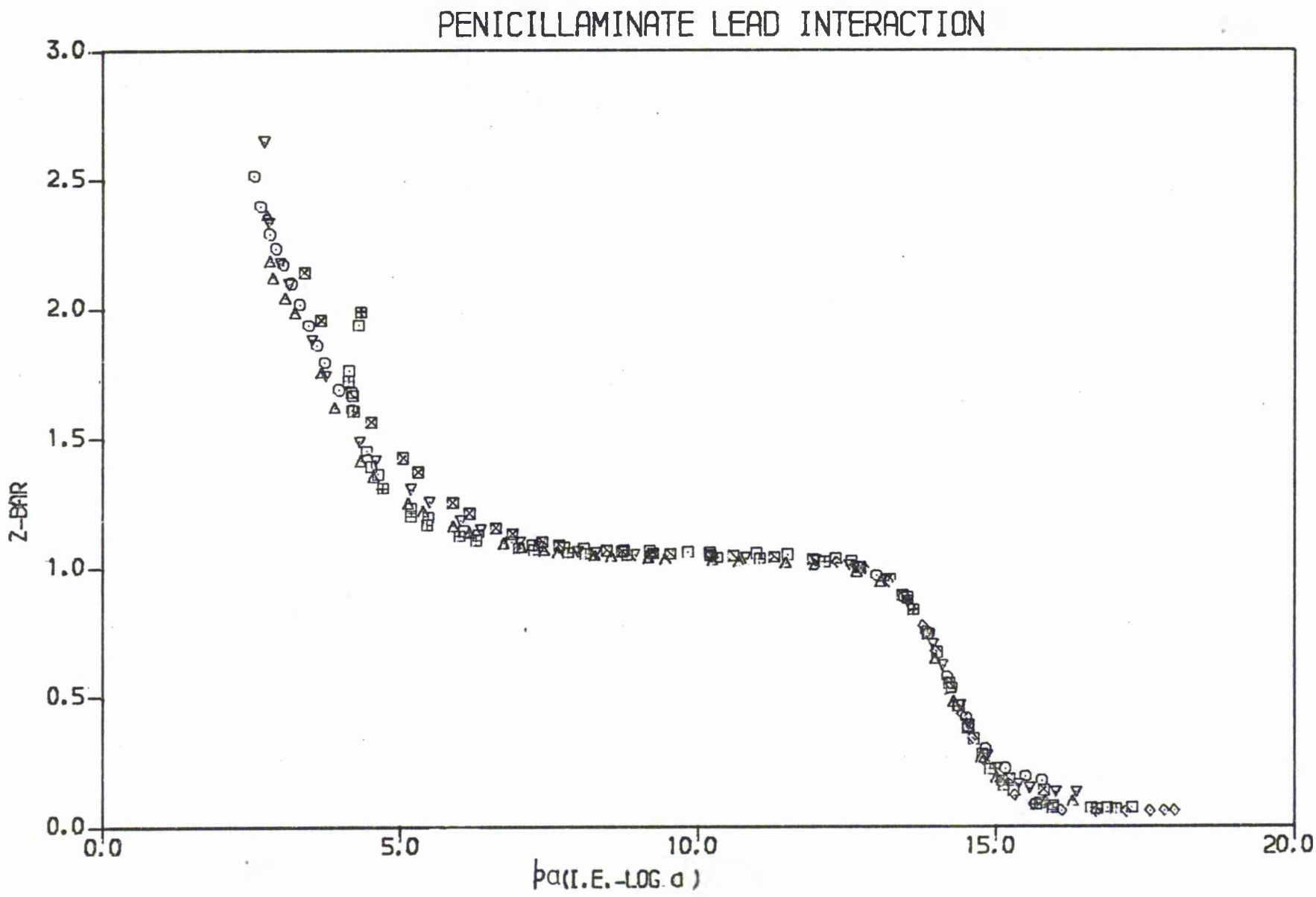


FIGURE 29 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 7

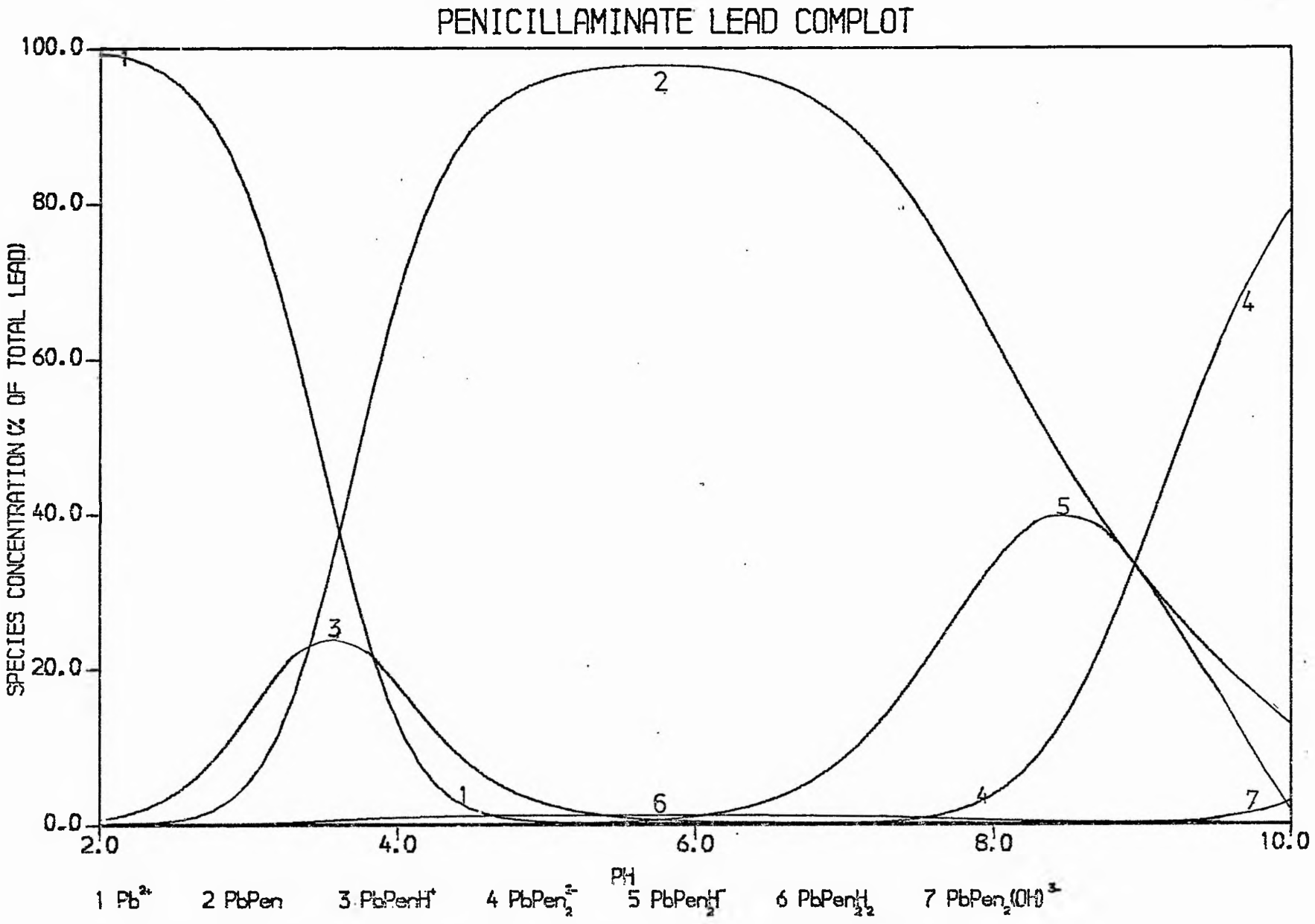


FIGURE 30 : COMPLOT USING THE FORMATION CONSTANTS FROM TABLE 7 PLUS LOG β_{212} = 34.0 AND LOG β_{21-1} = 7.551

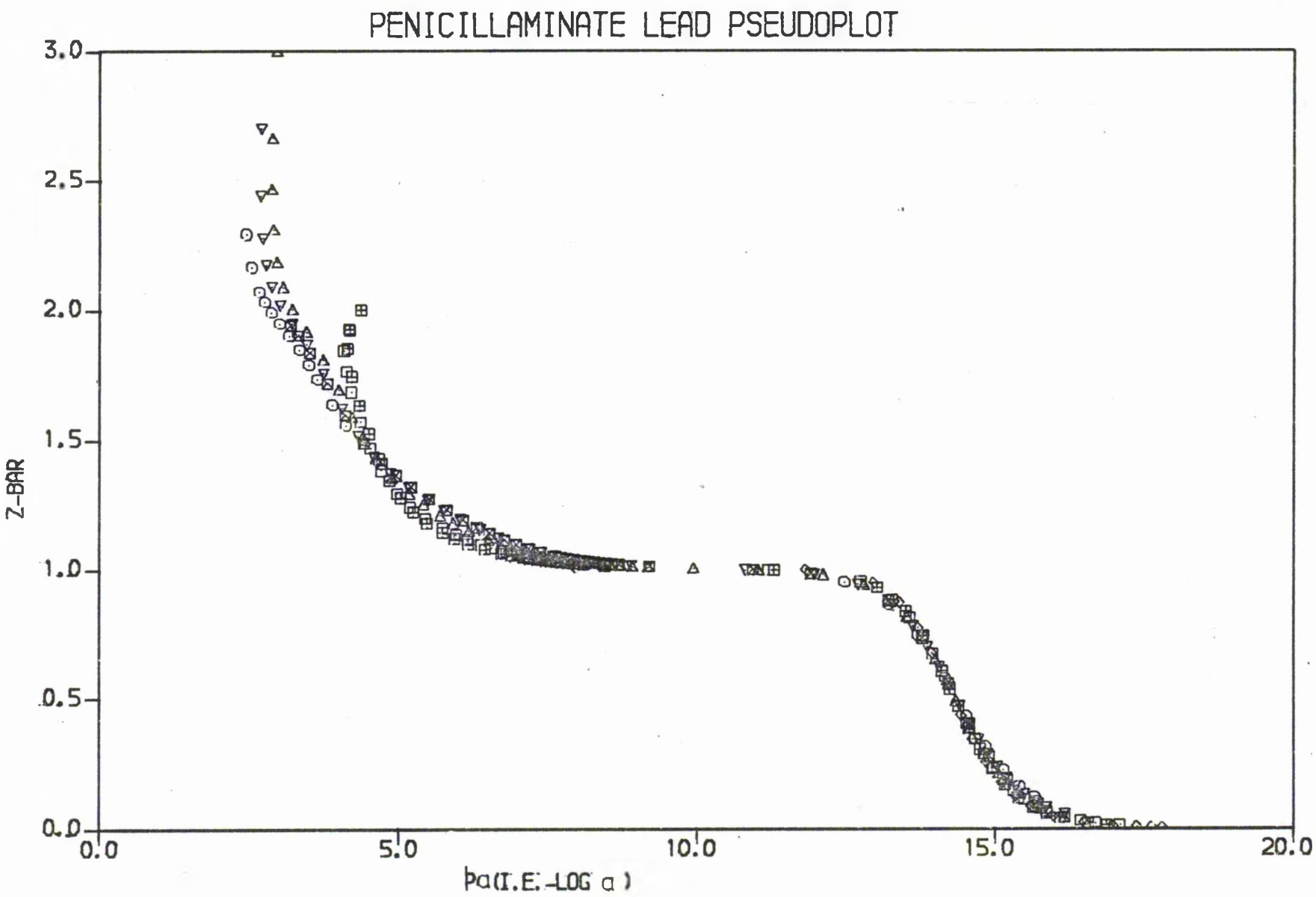


FIGURE 31 : PSEUDOPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

bonding in the 110 complex thus causing steric hindrance to forming the 210 complex. Thus we expect D-penicillaminate to be tridentate to lead(II).

LEAD(II)-GLUTATHIONATE

Experimental results are shown in appendix 2 - table 8 and in figure 32.

In order to avoid precipitation the lead(II) concentration had to be kept below about 2.5mM and a ligand : metal ratio of ≥4:1 had to be used. A sample of the precipitate, which occurs around pH4 was collected and analysed. The analysis was consistent with the 111 complex which would be uncharged and so might be insoluble. A COMPLOT model of the system, figure 33, shows that this is indeed the predominant species around pH4.

The species searched for were those with p 1 to 3; q 1, 2 and r -2 to 2, those giving convergence being 110, 210, 111, 211, 212 and 21-1 with log β values of 10.57, 15.00, 17.136, 24.664, 32.10 and 4.5 respectively. The inclusion of the 21-1 complex improves the sum of squared residuals only slightly and does not improve the PSEUDOPLOT fit. Also, figure 33 shows that this is only a minor species and then only at high pH. Thus the value determined for log β_{21-1} is not reliable.

A simulated set of formation curves obtained from PSEUDOPLOT using the formation constants from table 7 is shown in figure 34.

The log formation constant for the 110 complex reported here agrees well with the value of 10.6 found by Li and Manning [127]. No other workers have reported constants for the protonated species.

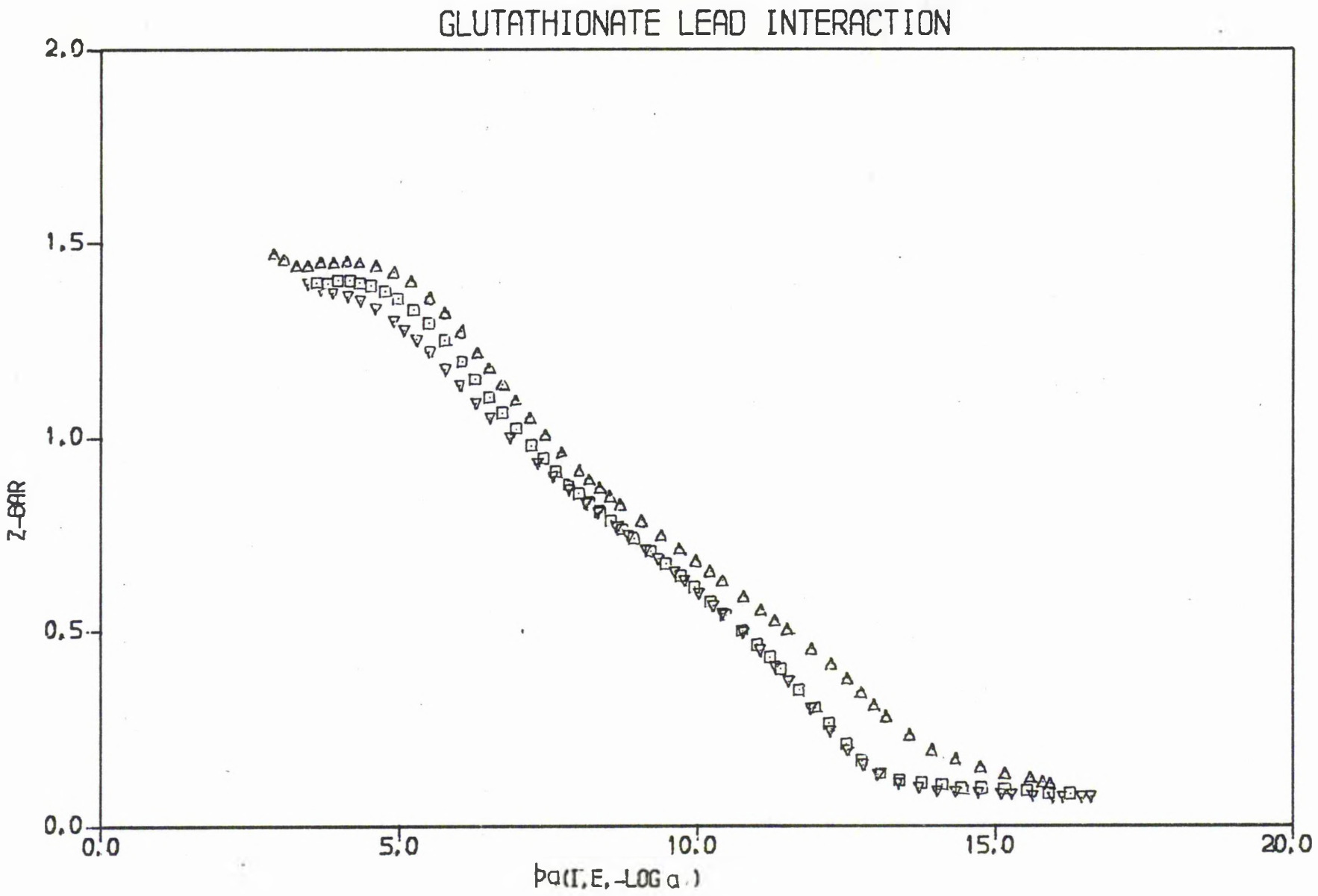


FIGURE 32 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 8

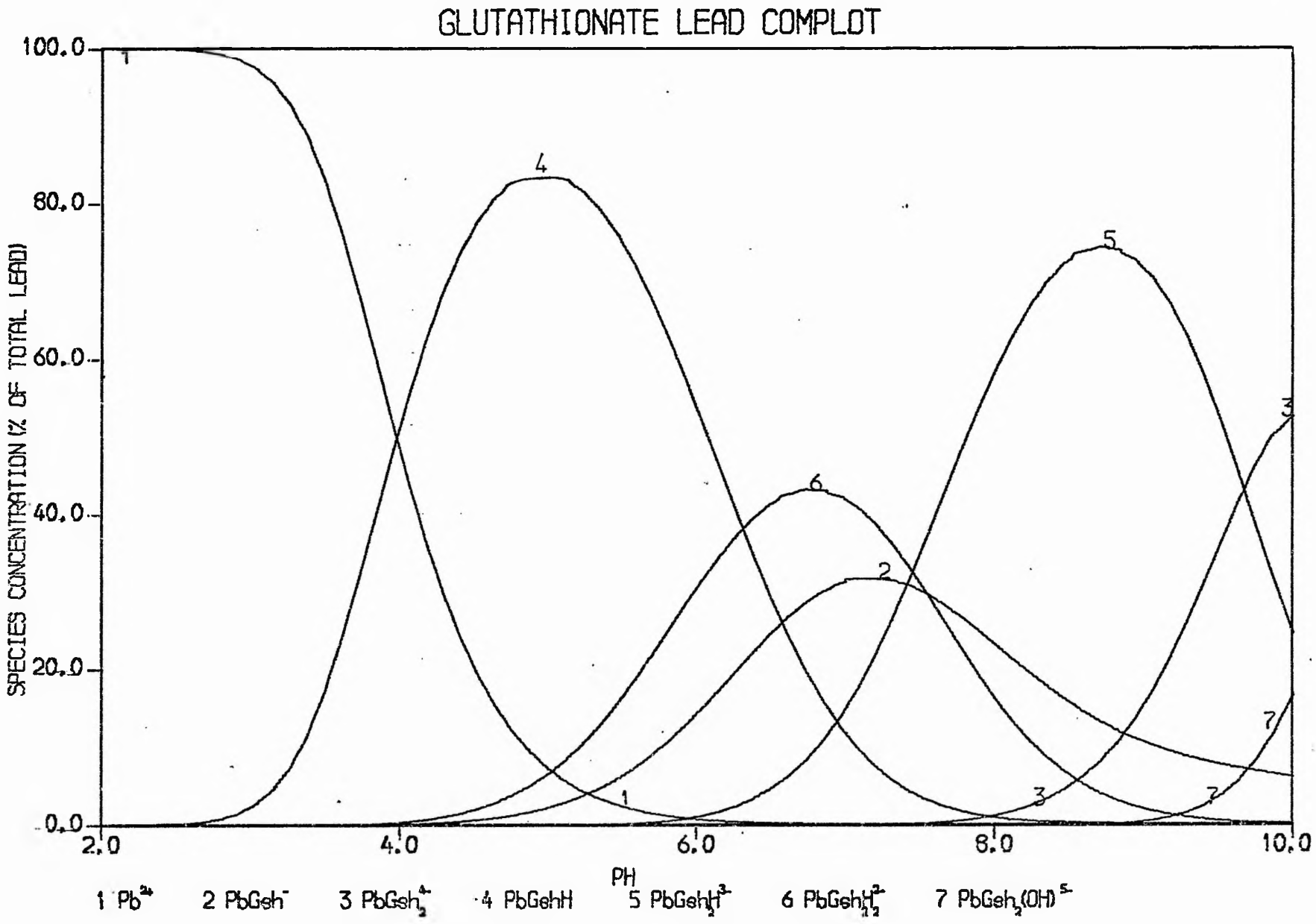


FIGURE 33 : COMPLOT USING THE FORMATION CONSTANTS FROM TABLE 7 PLUS LOG β_{21-1} = 4.5

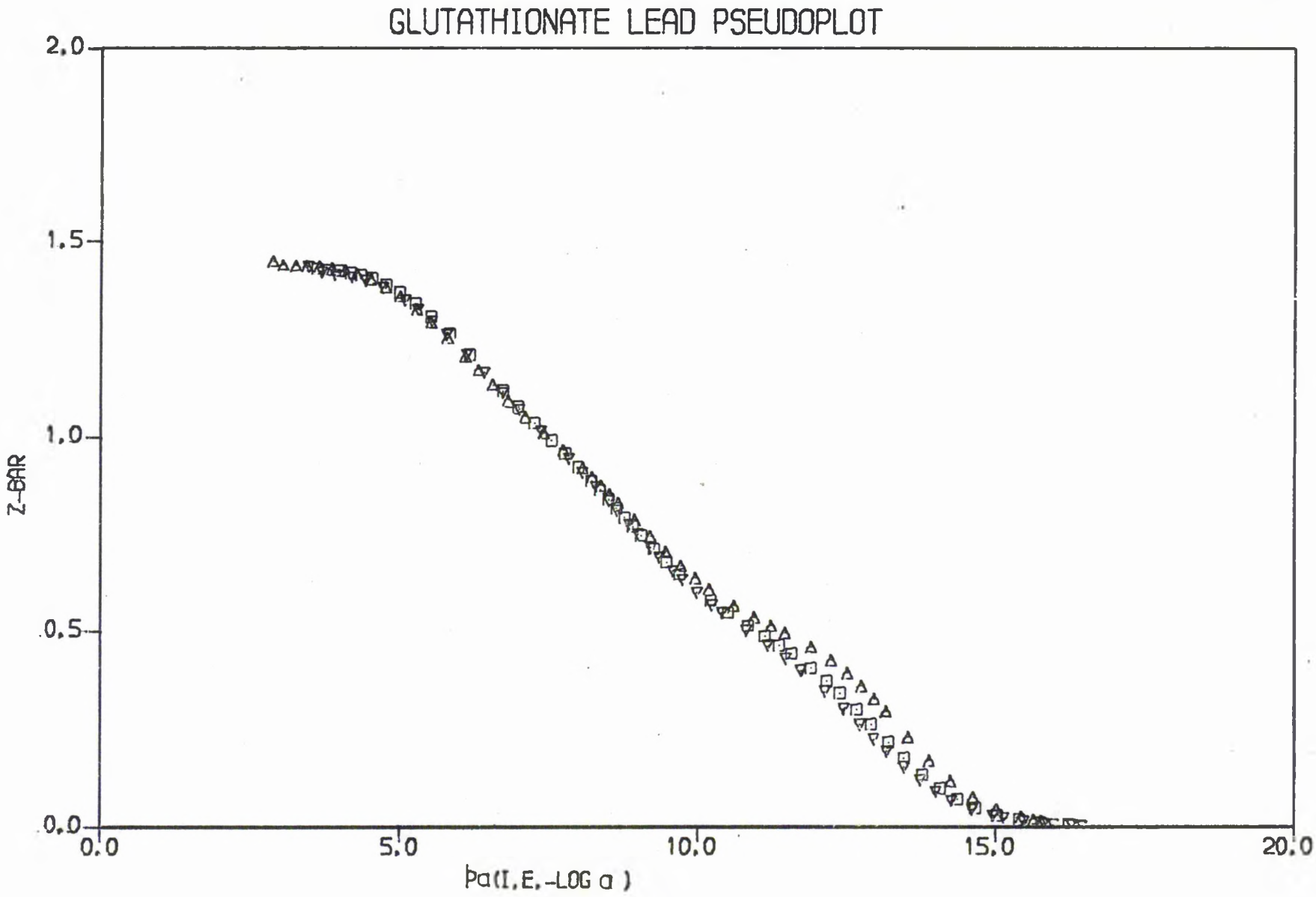


FIGURE 34 : PSEUDOPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

The ligand glutathionate has eight sites at which binding to a metal ion may occur and so more information is required before any predictions of structures can be made. Proposed structures for these complexes will be discussed in Chapter 8.

ZINC(II)-GLUTATHIONATE

Experimental results are shown in appendix 2 - table 9 and in figure 35.

The spreading of the formation curves indicates that protonated species are present in this case also. The complexes searched for were those with p 0 to 3; q 1, 2 and r -2 to 2 those giving convergence being 110, 210, 111, 11-1, 211, 212 and 21-1 with log β values of 8.568, 13.586, 14.762, -0.074, 23.271, 30.616 and 3.63 respectively. The complexes 11-1 and 21-1 have high standard deviations but their inclusion improves the PSEUDOPLOT fit at high $\bar{Z}$ and a COMPLOT model of the system (figure 36) shows that they would be present to a significant extent at higher pHs.

A simulated set of formation curves obtained from PSEUDOPLOT using the above constants is shown in figure 37.

Other workers have reported log β_{110} values ranging from 5.1 [140] to 8.30 [127] so again our values, at higher background ionic strength, are slightly higher. Perrin and Watt [114] report formation constants for the species 110, 210, 111, 11-1, 211, 212, 21-1 and 120 with which the values reported here are in general agreement considering that their working conditions were 37°C, I = 0.15MKNO_3. However, it was found

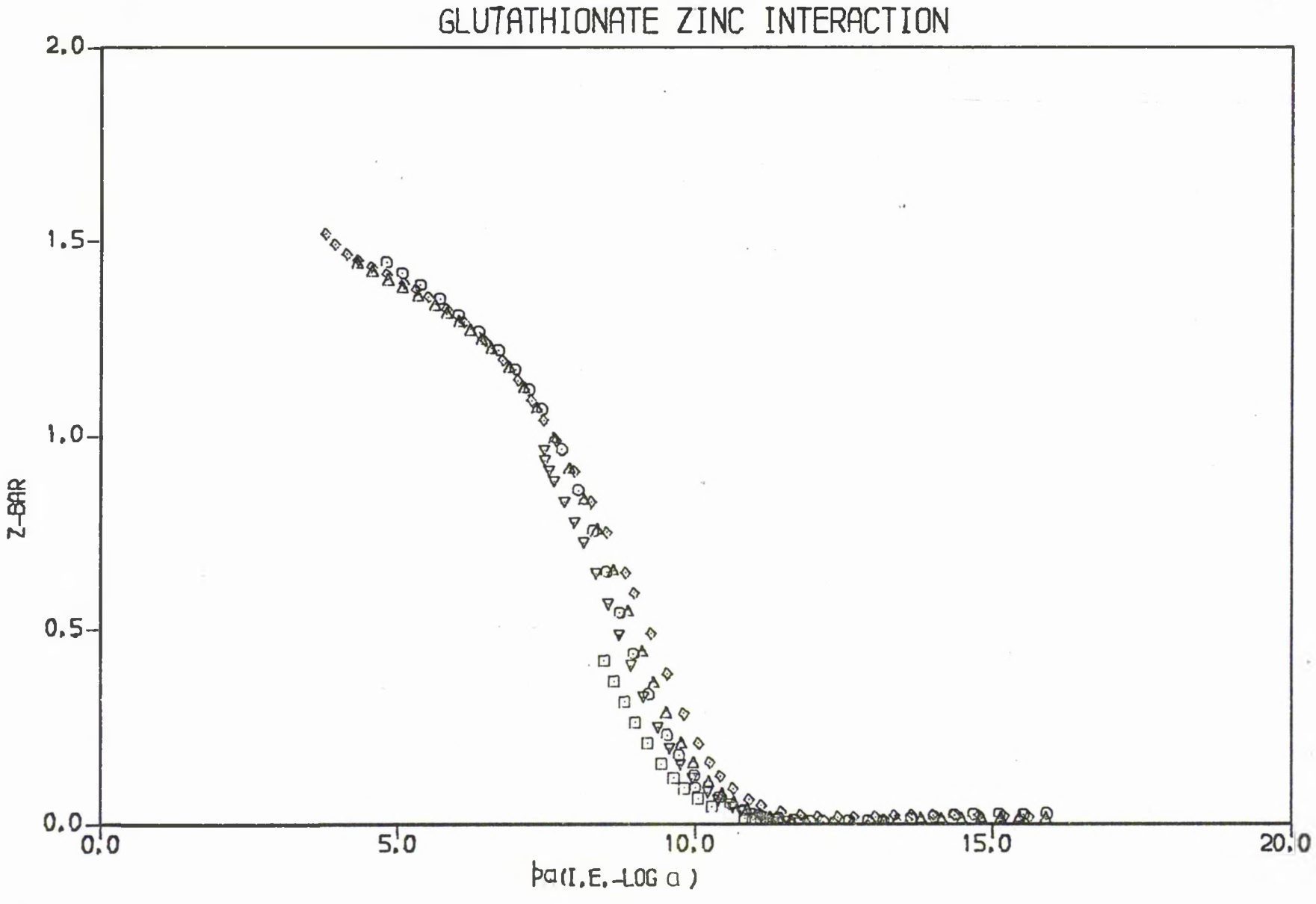


FIGURE 35 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 9

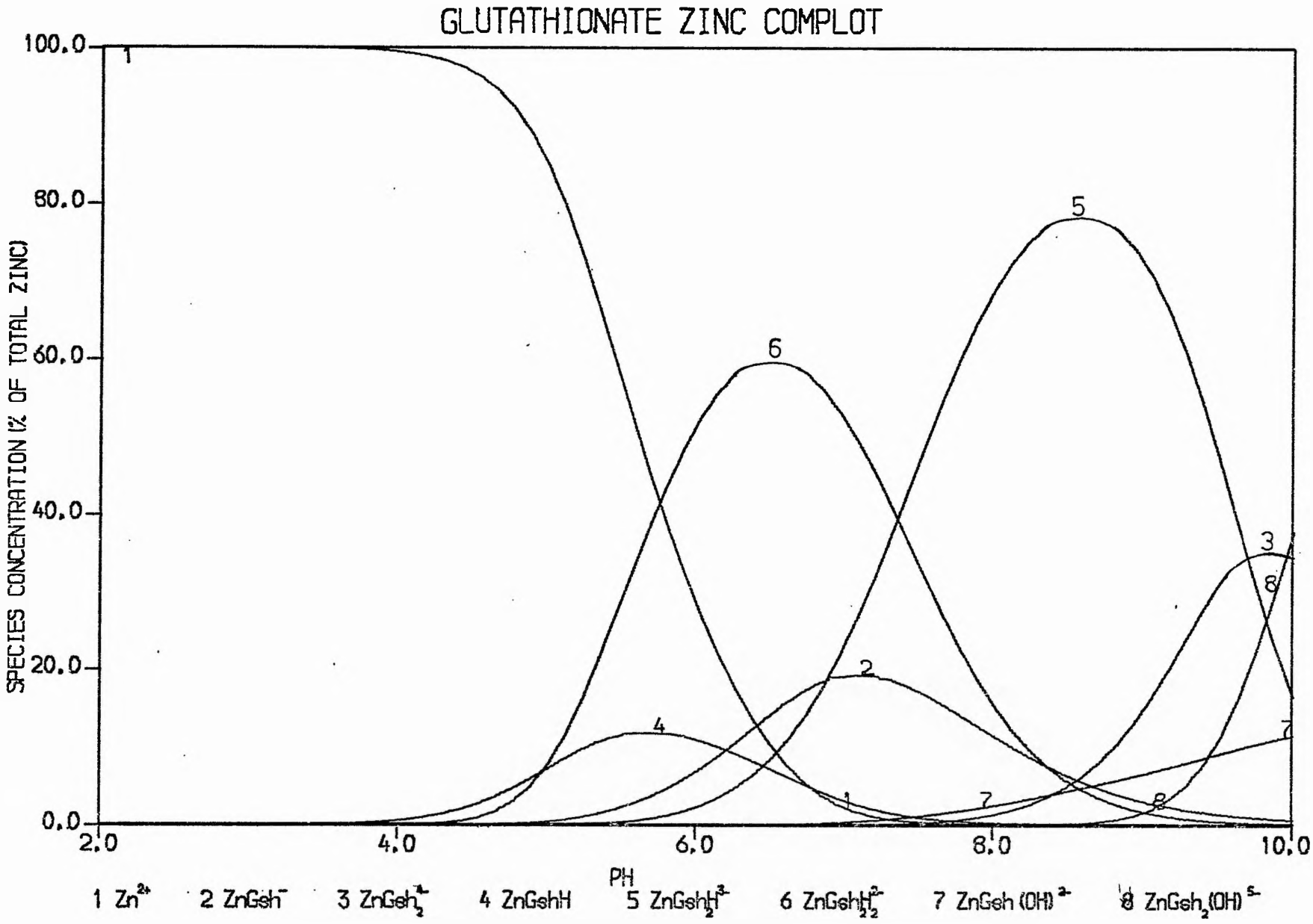


FIGURE 36 : COMPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

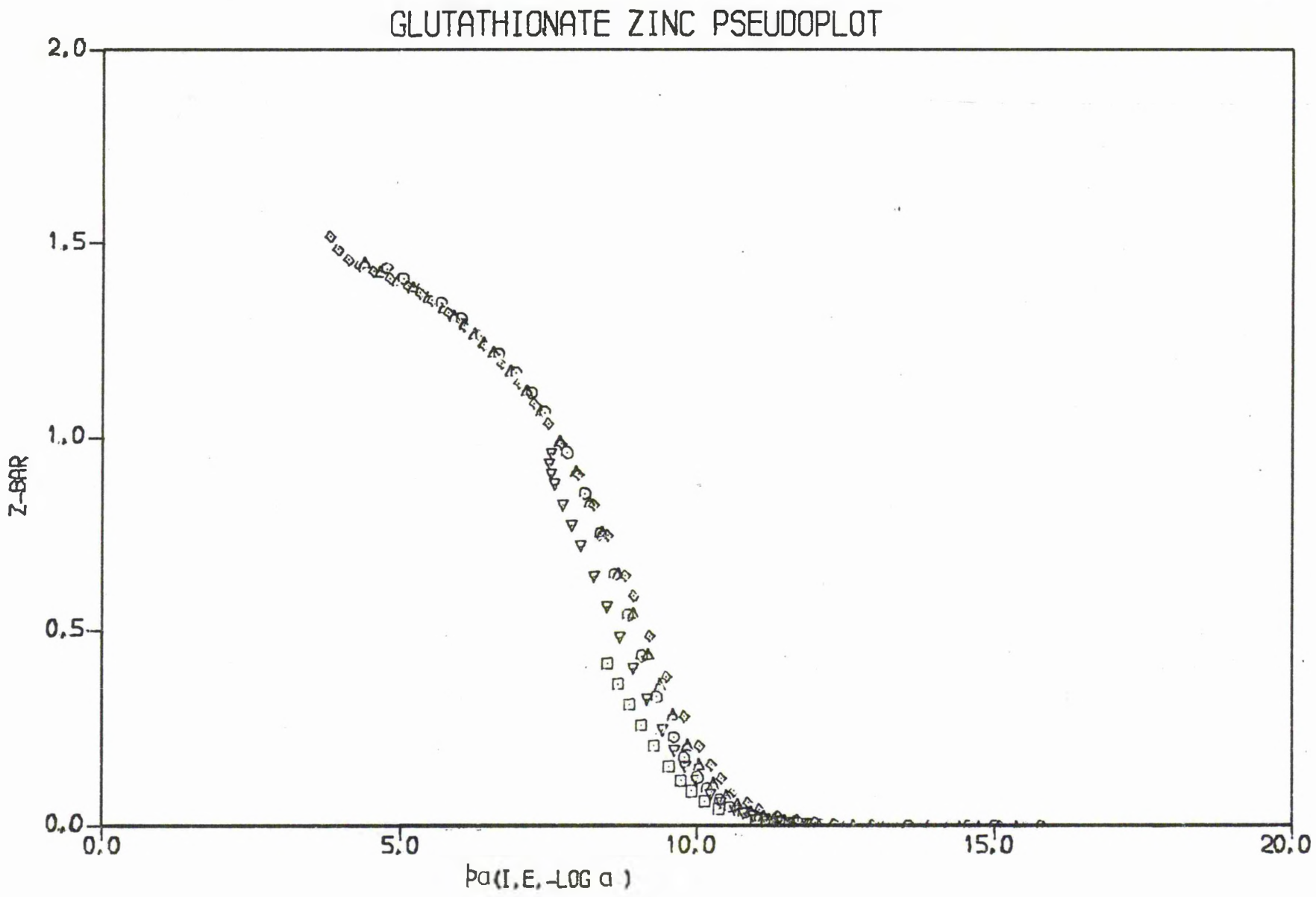


FIGURE 37 : PSEUDOPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

impossible, using either SCOGS or MINIQUAD, to refine a constant for the 120 species from our data.

From our formation constants one might postulate binding sites similar to those suggested by the Canberra workers [114] although the prediction of binding sites from formation constant values alone is an unreliable process for such a complicated ligand.

LEAD(II)-GLYCINATE

Experimental results are shown in appendix 2 - table 10 and in figure 38.

The formation curves only reach a $\bar{Z}$ of around 1 because of the precipitation of lead hydroxides at higher pHs. The spread of the curves is characteristic of hydroxy species and is caused mainly by the four lead hydroxy complexes $PbOH^{+}$, $Pb_4(OH)_4^{4+}$, $Pb_3(OH)_4^{2+}$ and $Pb_6(OH)_8^{4+}$.

The complexes searched for were those with p 1 to 3; q 1, 2 and r -4 to 2 those giving convergence being 110, 111 and 11-1 with log β values of 5.600, 11.40 and -2.142 respectively. A simulated set of formation curves obtained from PSEUDOPLOT using these constants is shown in figure 39.

Other workers have reported [65] formation constants for the 110 and 210 complexes but not for the protonated or hydroxy species. In general their values are lower than those reported here.

Proposed structures for these complexes will be discussed, in the light of thermodynamic data, in Chapter 8.

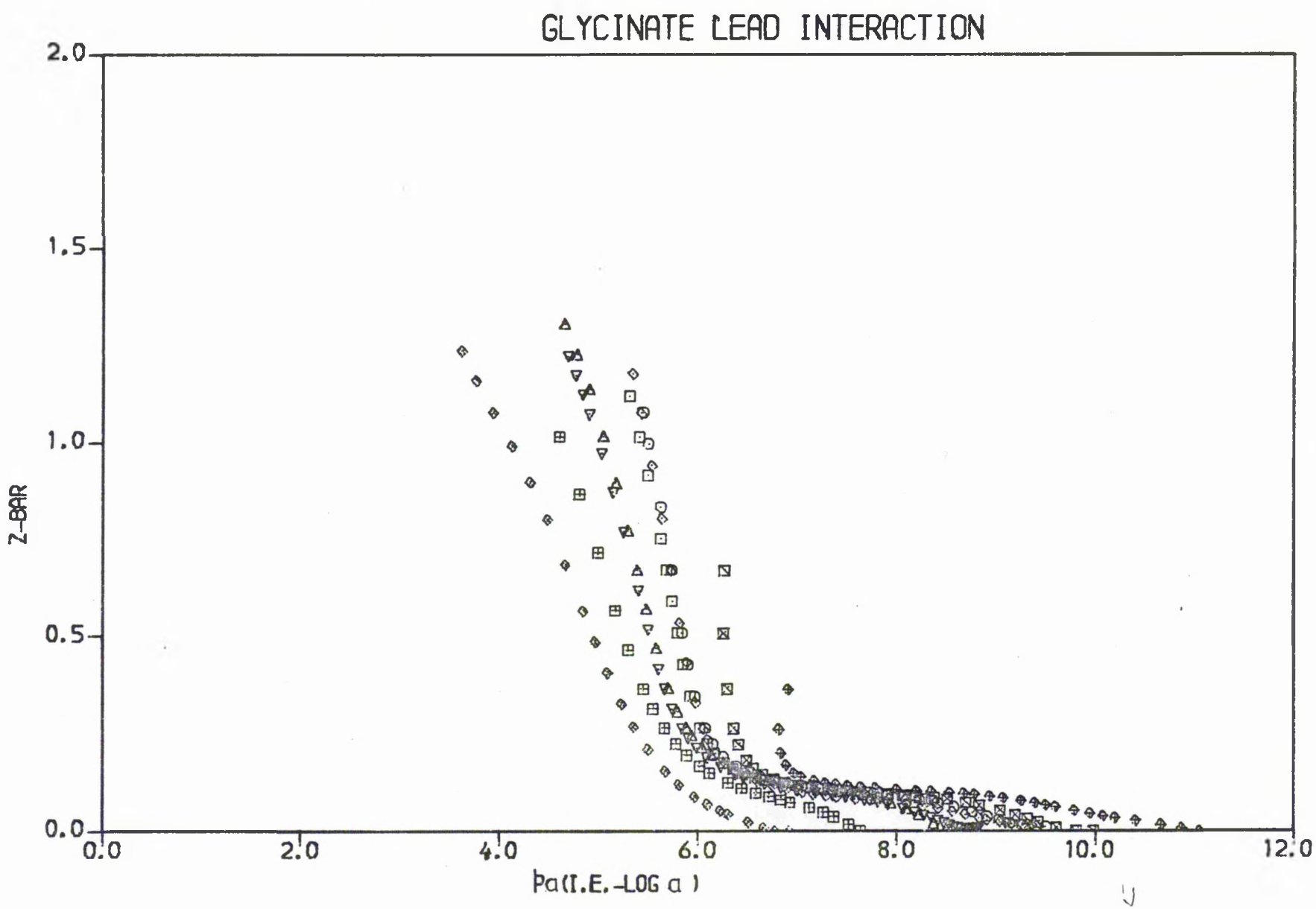


FIGURE 38 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 10

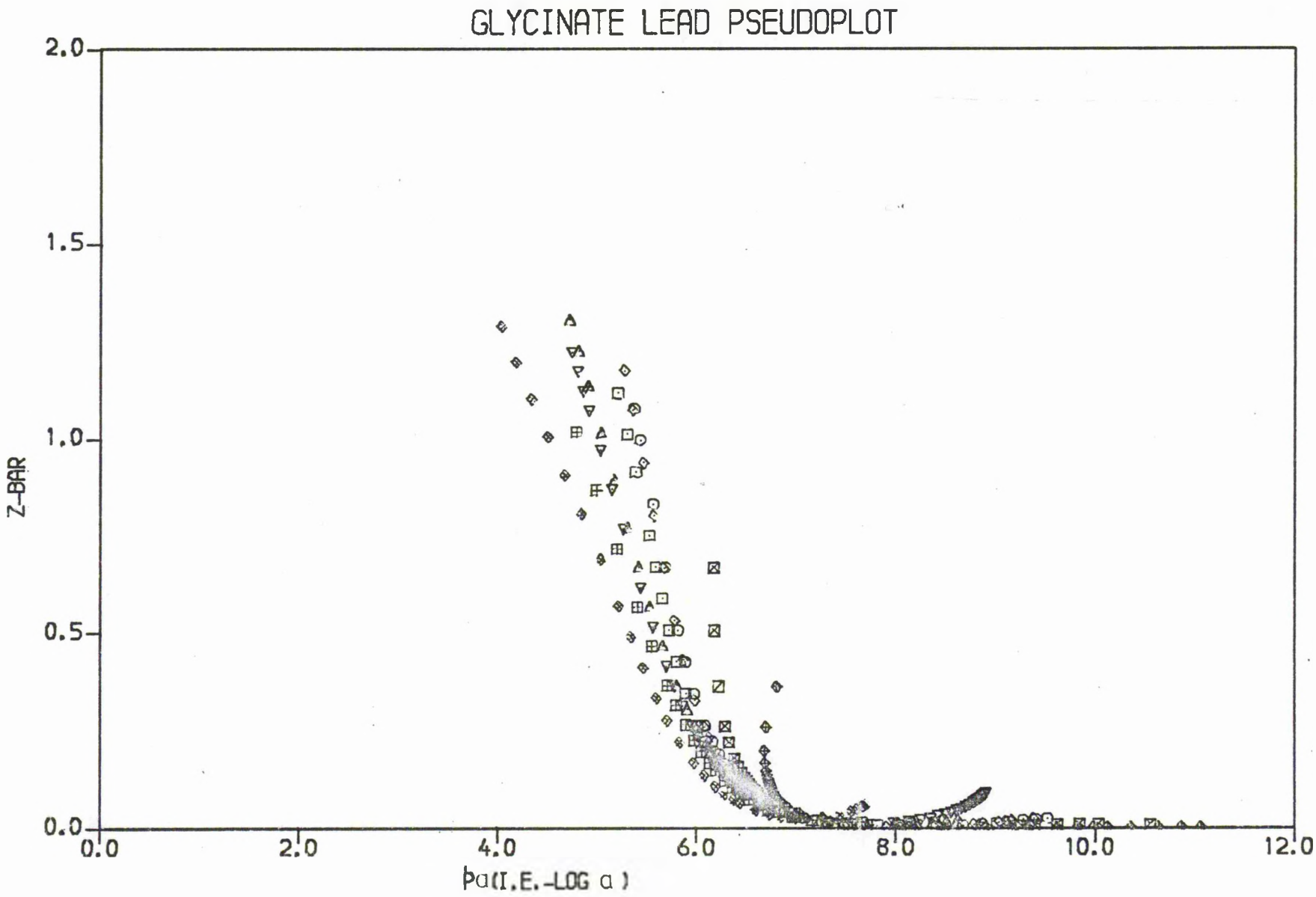


FIGURE 39 : PSEUDOPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

LEAD(II)-GLYCYLGLYCINATE

Experimental data are shown in appendix 2 - table 11 and in figure 40.

Again a marked pattern of formation curves is obtained the curve position depending both on the metal concentration and on the ligand : metal ratio.

The complexes searched for were those with p 1, 2; q 1, 2 and r -2 to 2 those giving convergence being 110 and 111 with log β values of 3.375 and 9.907 respectively. In this case the inclusion of a 11-1 species prevented convergence in either SCOGS or MINIQUAD. A simulated set of formation curves obtained from PSEUDOPLOT using these constants is shown in figure 41.

For this ligand, log formation constants for the protonated species have been reported by other workers these being 9.71 [141] and 9.51 [122] determined using polarography and nuclear magnetic resonance spectroscopy respectively. Our work is in general agreement with these and other literature values [65] considering the different conditions of temperature and background ionic strength.

Proposed structures for these complexes will be discussed in Chapter 8.

LEAD(II)-GLYCYLGLYCYLGLYCINATE

Experimental results are shown in appendix 2 - table 12 and in figure 42.

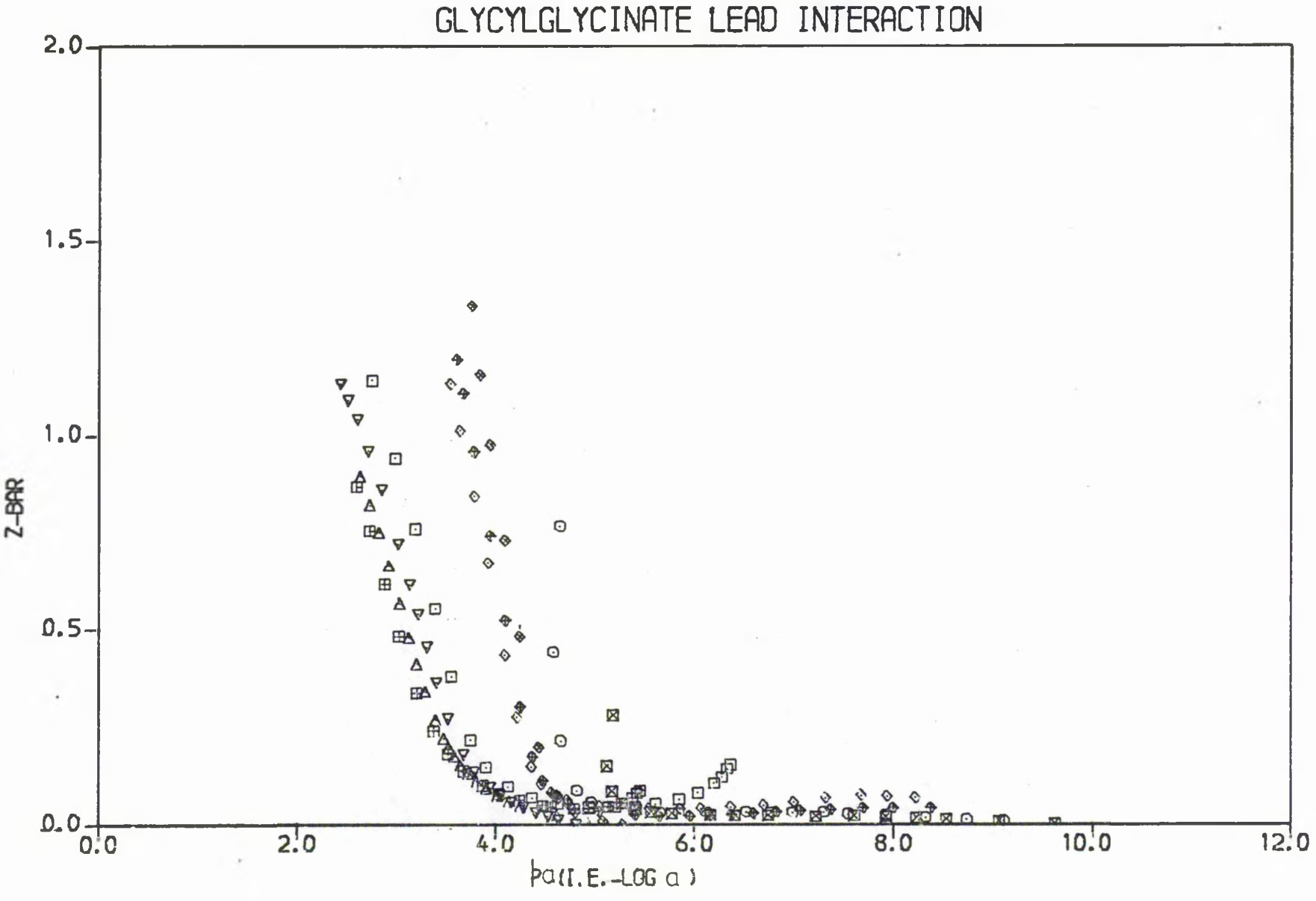


FIGURE 40 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 11

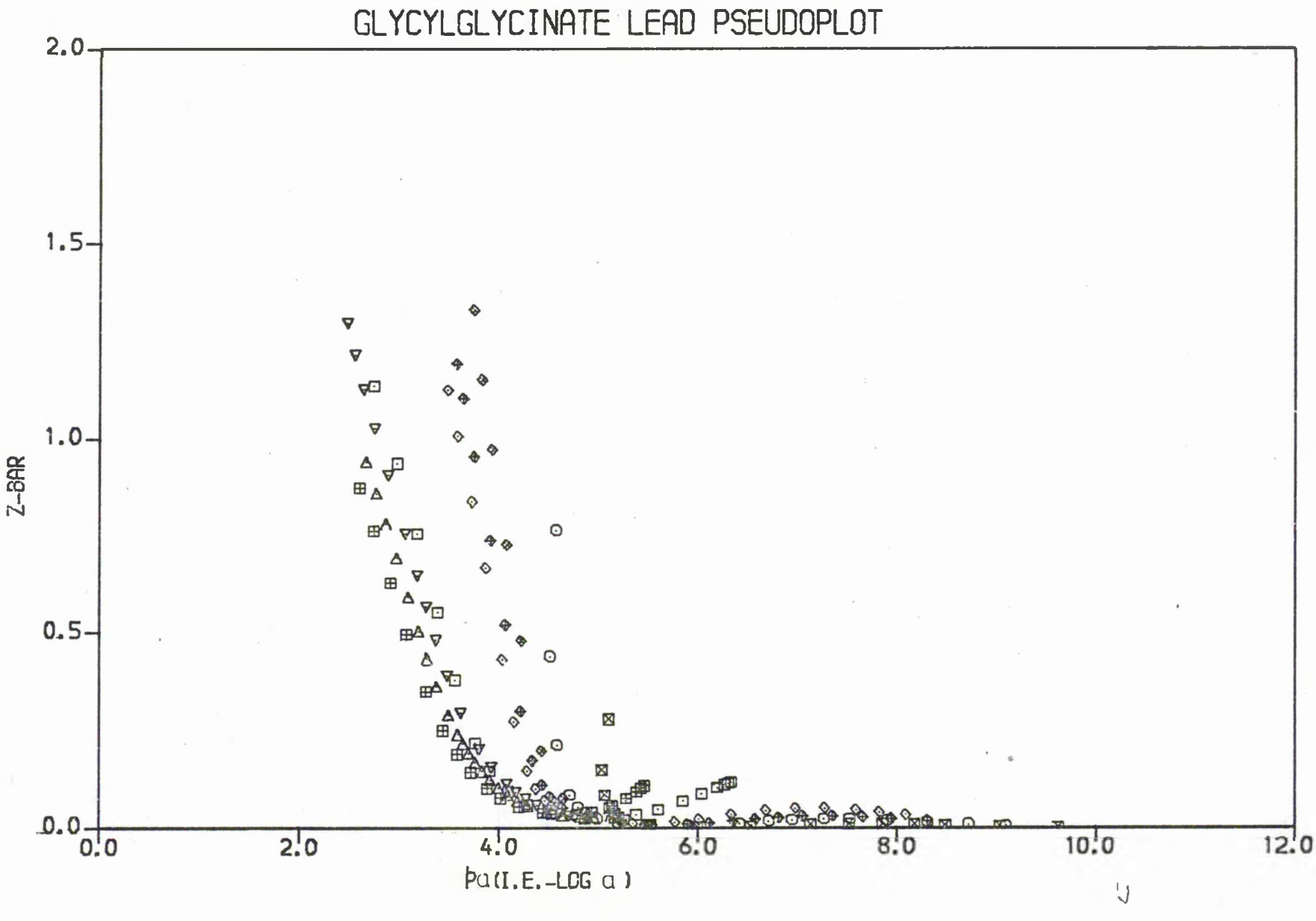


FIGURE 41 : PSEUDOPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

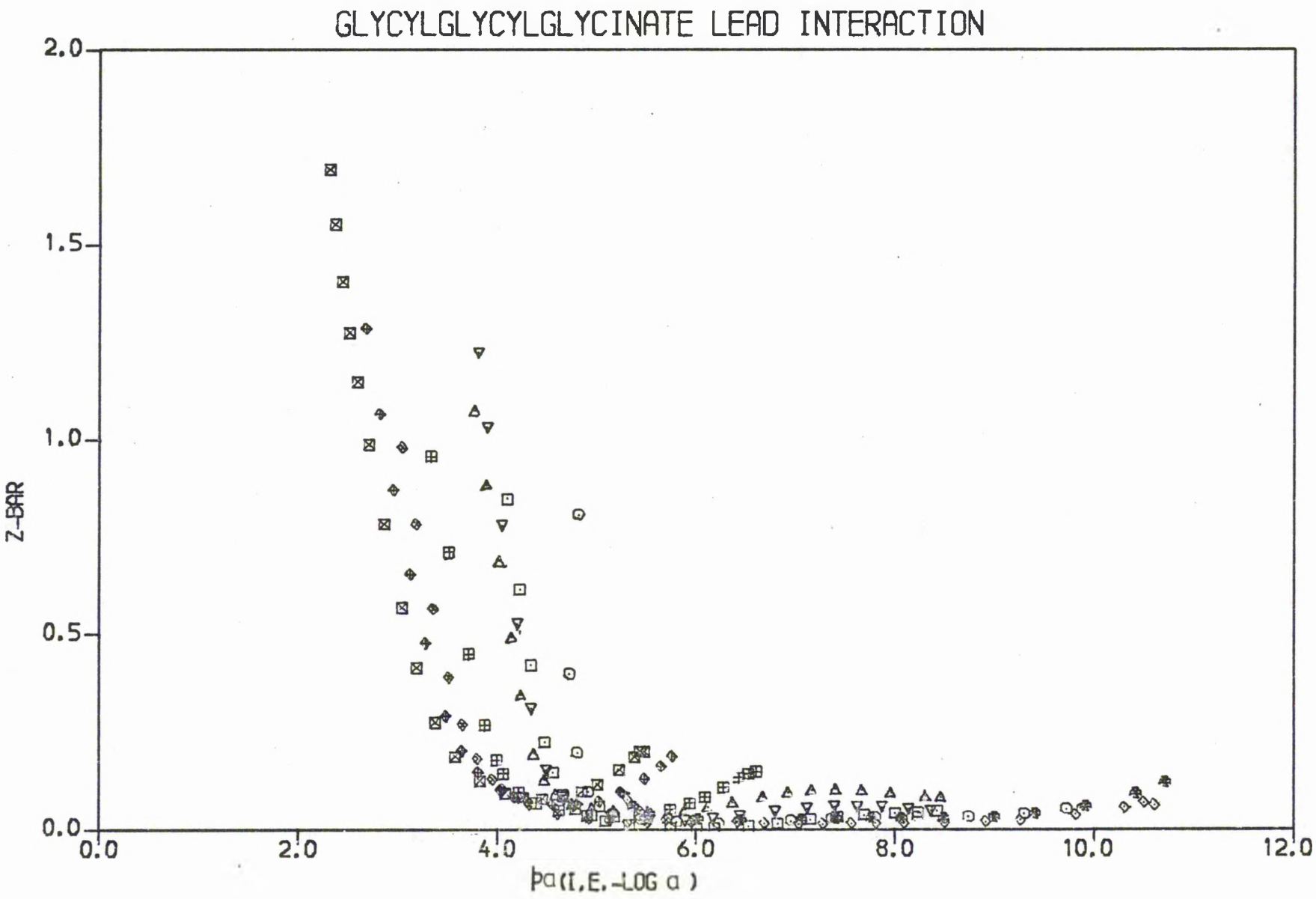


FIGURE 42 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 12

The complexes searched for were those with p 1, 2; q 1, 2 and r -2 to 2 those giving convergence being 110, 111 and 11-1 with log β values of 3.767, 10.403 and -3.762 respectively. A simulated set of formation curves obtained from PSEUDOPLOT using these constants is shown in figure 43.

A value for log β_{111} of 9.54 has been reported [122] as well as formation constants for the 110 and 210 species [65] but no literature value is available for the hydroxy complex.

Proposed structures for these complexes will be discussed in Chapter 8.

LEAD(II)-CHLORIDE

For the study of the interaction of chloride ions with lead(II), a chloride sensitive electrode was used and a silver/silver chloride reference cell.

Ag(s)-AgCl(s)	solution under study I = 3.00M $(Na^+)ClO_4^-$	3.00M $NaClO_4$	AgCl(s)-Ag(s), 0.100M NaCl I = 3.00M $(Na^+)ClO_4^-$

This electrode pair gives a potential described by $E=E_o - \frac{RT}{F} \ln[Cl^-]$ and was calibrated by the measurement of E for solutions of known $[Cl^-]$. A calibration line is thus constructed, see table 6 and figure 44 for the experimental data, which can be extrapolated to $-\log[Cl^-] = 0$ using a simple least-squares procedure in order to obtain the value of E_o. This E_o value is checked before and after each titration using solutions of known chloride concentration.

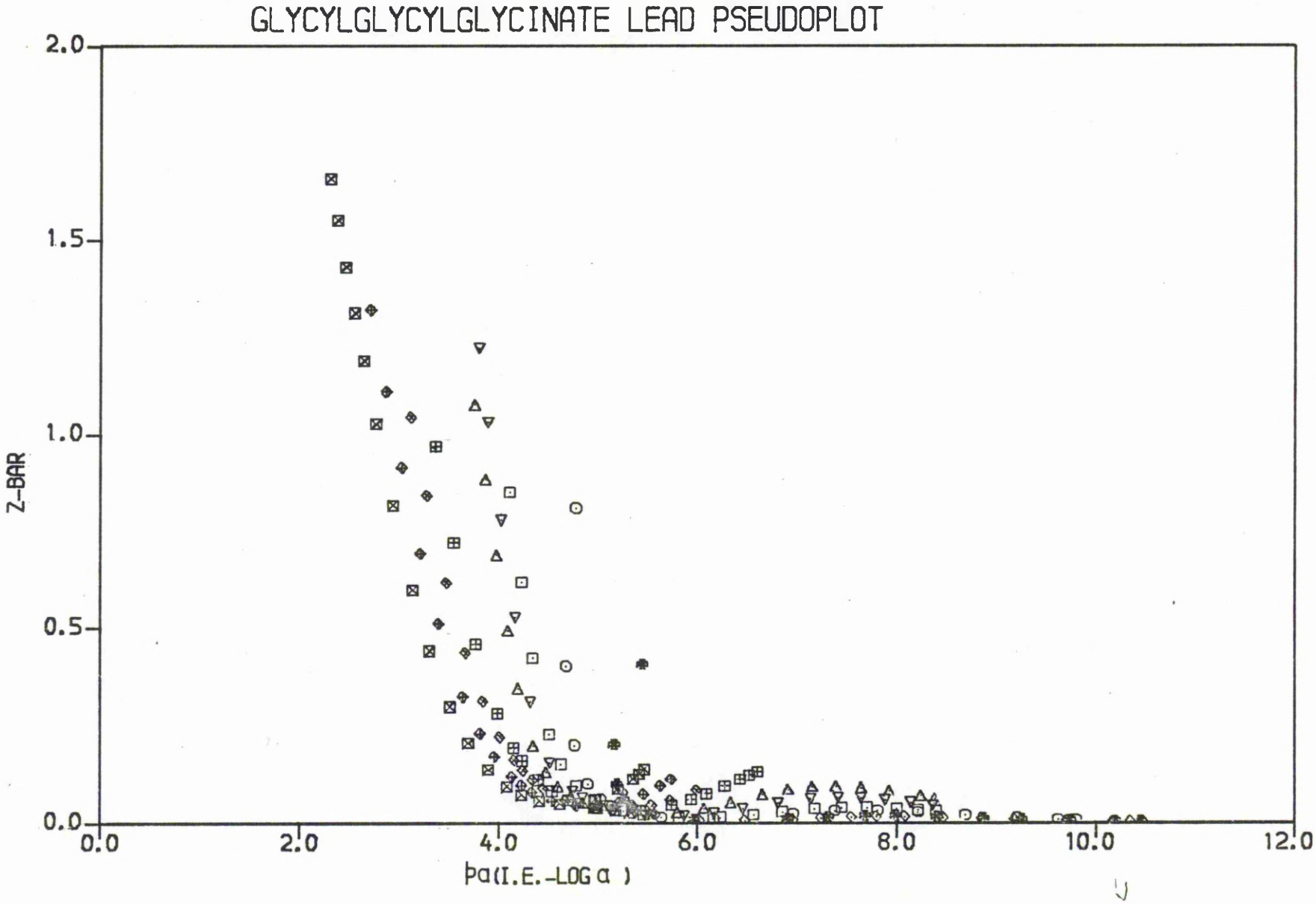


FIGURE 43 : PSEUDOPLOT USING THE FORMATION CONSTANTS FROM TABLE 7

TABLE 6

EXPERIMENTAL DATA FOR THE CALIBRATION OF THE CHLORIDE SENSITIVE ELECTRODE

Titrate : 20.00ml of 3.00M $NaClO_4$

Titrant : 19.92mM NaCl, I = 3.00M$(Na^+)ClO_4^-$

Titre (ml)	E (mV)	$-\log[Cl^-]$	Titre (ml)	E (mV)	$-\log[Cl^-]$
1.10	-106.1	2.98	7.70	-69.4	2.26
1.40	-101.0	2.88	9.20	-66.6	2.20
1.80	-95.9	2.78	12.30	-62.6	2.12
2.20	-91.9	2.70	14.50	-60.4	2.08
2.70	-88.0	2.63	17.50	-58.1	2.03
3.40	-83.7	2.54	23.50	-54.8	1.97
4.20	-79.9	2.46	26.50	-53.7	1.95
5.20	-76.0	2.39	30.50	-52.3	1.92
6.20	-73.0	2.33			

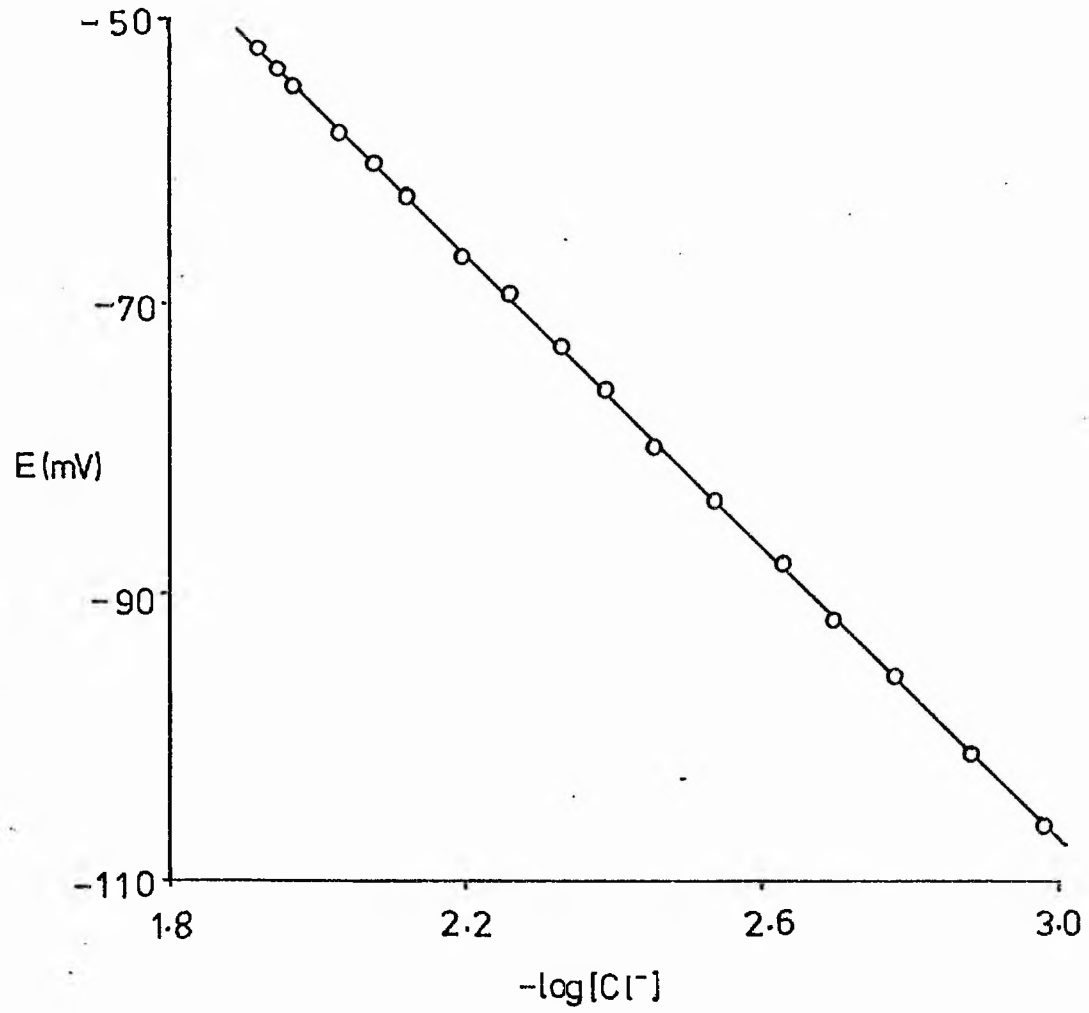


FIGURE 44 : CHLORIDE SENSITIVE ELECTRODE CALIBRATION

Experimental results for the interaction of lead(II) with chloride ions are shown in appendix 2 - table 13 and in figure 45.

Non-superimposable formation curves are found, their position depending on the lead(II) concentration.

The working pH range was 2.0 to 2.3 and so inclusion of Olin's four lead-hydroxy species in the calculations was not necessary. The complexes searched for were those with p 1 to 4; q 1 to 3 and r -2 to 0 and those giving convergence were 110, 210, 410, 11-1 and 41-1 with log β values of 1.14, 2.602, 5.410, -0.977 and 2.943 respectively. The HALTAFALL program was used to calculate the equilibrium concentrations of species present during the titrations and shows that all of the above complexes are significant.

Formation constants are reported in the literature [65] for the species 110 to 610 the values of which are generally lower than those reported here and solubility products are given [65] for the species 11-1. Haight and Peterson [142] have shown, from solubility and spectroscopic work, that lead(II) has a preference for even numbers of chloride ligands and this is borne out by our inability to determine a formation constant for the 310 complex.

The lead(II)-glycinate system was also examined again in the presence of chloride ions in order to determine whether any ternary lead(II)-glycinate-chloride complexes could be detected. However, no ternary species were found since as the pH is raised to allow lead(II)-glycinate complex formation then the chloride ion is displaced from the lead(II) by hydroxide in order to form either binary lead(II)-hydroxy species or ternary lead(II)-glycinate-hydroxy species.

The results from the metal-ligand interaction work are summarised in table 7.

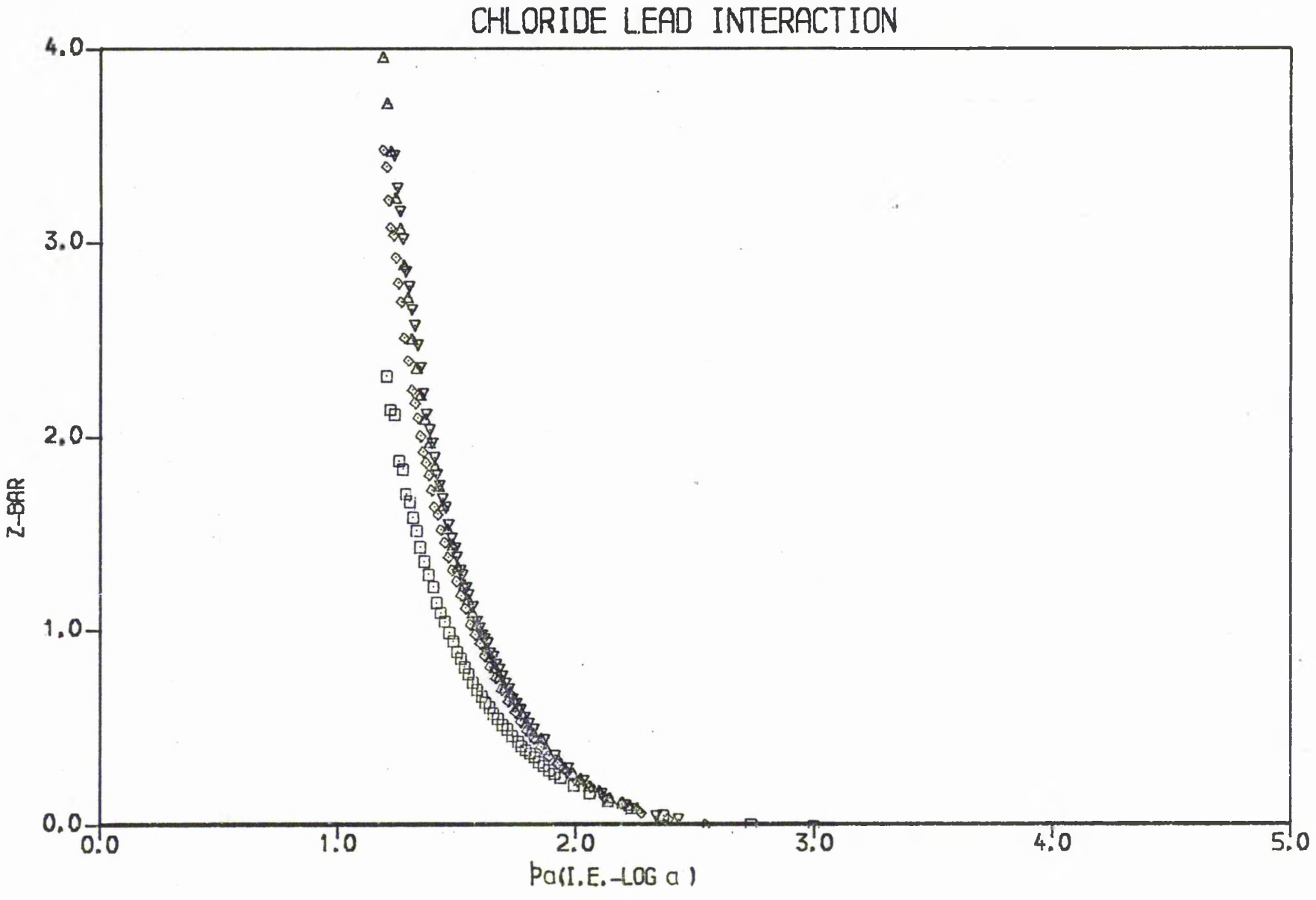


FIGURE 45 : ZPLOT OF THE DATA FROM APPENDIX 2 - TABLE 13

TABLE 7

Log formation constants for ligand anion-metal-proton interactions at 25°C, I = 3.00M(Na^+)ClO_4^-

	pqr	log β_{pqr}	n*
Lead(II)-aspartate	110	6.878 ± 0.021	198
	210	10.014 ± 0.044	
	111	12.695 ± 0.030	
	112	16.167 ± 0.053	
	211	19.181 ± 0.046	
	212	24.985 ± 0.045	
Lead(II)-cysteinate	110	13.213 ± 0.016	137
	210	18.571 ± 0.045	
	111	17.347 ± 0.053	
	211	27.476 ± 0.043	
Zinc(II)-cysteinate	210	19.395 ± 0.019	218
	211	25.856 ± 0.058	
	212	31.879 ± 0.057	
	430	46.247 ± 0.095	
	431	52.50 ± 0.10	
Lead(II)-glutamate	110	5.344 ± 0.027	146
	111	12.173 ± 0.018	
Lead(II)-ethylenediamine-tetraacetate	110	15.186 ± 0.078	272
	111	18.010 ± 0.069	

Table 7 continued.

Zinc(II)-ethylenediamine-	110	14.873 ± 0.050	335
tetraacetate	111	17.965 ± 0.034	
Lead(II)-D-penicillaminate	110	14.321 ± 0.023	199
	210	19.049 ± 0.050	
	111	17.723 ± 0.053	
	211	27.978 ± 0.074	
Lead(II)-glutathionate	110	10.57 ± 0.19	151
	210	15.00 ± 0.23	
	111	17.136 ± 0.034	
	211	24.664 ± 0.071	
	212	32.10 ± 0.11	
Zinc(II)-glutathionate	110	8.568 ± 0.015	168
	210	13.586 ± 0.098	
	111	14.762 ± 0.058	
	11-1	-0.074 ± 0.054	
	211	23.271 ± 0.017	
	212	30.616 ± 0.018	
	21-1	3.63 ± 0.27	
Lead(II)-glycinate	110	5.600 ± 0.034	289
	111	11.40 ± 0.13	
	11-1	-2.142 ± 0.035	

Table 7 continued

Lead(II)-glycylglycinate	110	3.375 ± 0.039	176
	111	9.907 ± 0.032	
Lead(II)-glycylglycylglycinate	110	3.767 ± 0.039	192
	111	10.403 ± 0.037	
	11-1	-3.762 ± 0.040	
Lead(II)-chloride	110	1.14 ± 0.11	200
	210	2.602 ± 0.093	
	410	5.410 ± 0.046	
	11-1	-0.977 ± 0.039	
	41-1	2.943 ± 0.069	

* n = number of experimental observations

CHAPTER 6

RESULTS - THERMODYNAMIC FUNCTIONS

CONTENTS

CHAPTER 6

RESULTS - THERMODYNAMIC FUNCTIONS

Calorimetry

All calorimetric work was carried out at 25°C, I = 3.00M(Na^+)ClO_4^- using the apparatus described in Chapter 4.

CALORIMETER CALIBRATION

For each volume of solution under study a calibration constant, E, is required so that the measured temperature rise, ΔT, can be converted into the quantity of heat, E, liberated by the chemical reactions. Thus, for a series of volumes, a known amount of electrical energy is added from a heating coil (electrical energy = voltage x current x time for which the current flows) and the temperature rise is measured. A calibration line is then plotted i.e. $E/\Delta T$ *versus* volume, which can be analysed by a least-squares procedure to give the 'best' value of the calibration constant for each volume. For an example of the experimental data see table 8 and figure 46.

TABLE 8

EXPERIMENTAL DATA FOR CALORIMETER CALIBRATION

Volume (ml)	$E/\Delta T$ ($J\ K^{-1}$)	Volume (ml)	$E/\Delta T$ ($J\ K^{-1}$)
100.57	486.7	123.57	605.5
103.57	507.4	126.57	611.8
107.57	524.4	130.57	640.7
111.57	543.0	134.57	655.6
114.57	551.9	138.57	668.6
117.57	564.3	142.57	681.2
120.57	580.2		

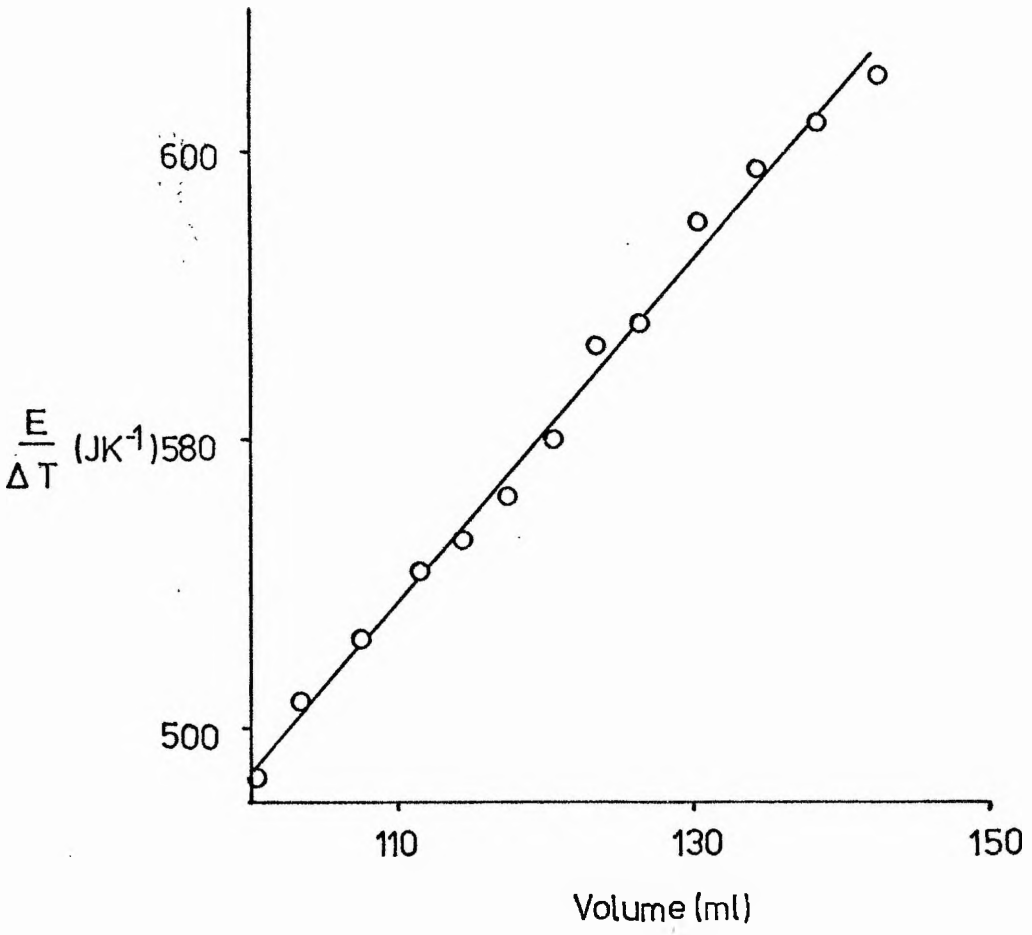


FIGURE 46 : CALORIMETER CALIBRATION LINE

TOLERANCES

In general, experimental errors, some values of which are shown in table 9, are ≤0.1% of the typical measured values.

TABLE 9

EXPERIMENTAL ERRORS

Calibration voltage	± 0.001 V
Calibration current	± 0.0001 A
Calibration time	± 0.01s
Room Temperature	± 0.5°C
Bath Temperature	± 0.0005°C
Temperature Change	± 0.00005°C

EXPERIMENTAL PROCEDURE

The reaction vessel is charged with the titrate from a silicone lined flask, calibrated to deliver 99.57ml, and is allowed to equilibrate in the water bath overnight. About two hours before starting a titration, the stirrer and the quartz thermometer are switched on and the system allowed to reach a steady state.

For each point in the titration we allow a 10 minute fore period, a 6 minute reaction period (after the addition of about 3ml of titrant) and a 10 minute aft period. Between titration points the solution is cooled so that each addition is made at the same temperature. Since the fore and aft periods show approximately the same variation of temperature with time, they are simply extrapolated to the half reaction time in order to calculate the temperature rise.

The composition of the system for each titration point is calculated using the program PSEUDOPLOT and so the change in the number of moles of each species caused by the titrant addition can be determined.

GLYCINATE PROTONATION

Experimental data are shown in appendix 3 - table 1 and enthalpic curves in figure 47.

If only simple species are present then the enthalpic curve should be independent of the concentration of ligand used [143]. The curves found are parallel but not superimposable, this being because each titration has a different initial $\bar{Z}$. If a correction is made for this, then very good superimposability is found up to $\bar{Z} = 1.0$. Above this, however, there is some scatter due to the enthalpy of protonation of

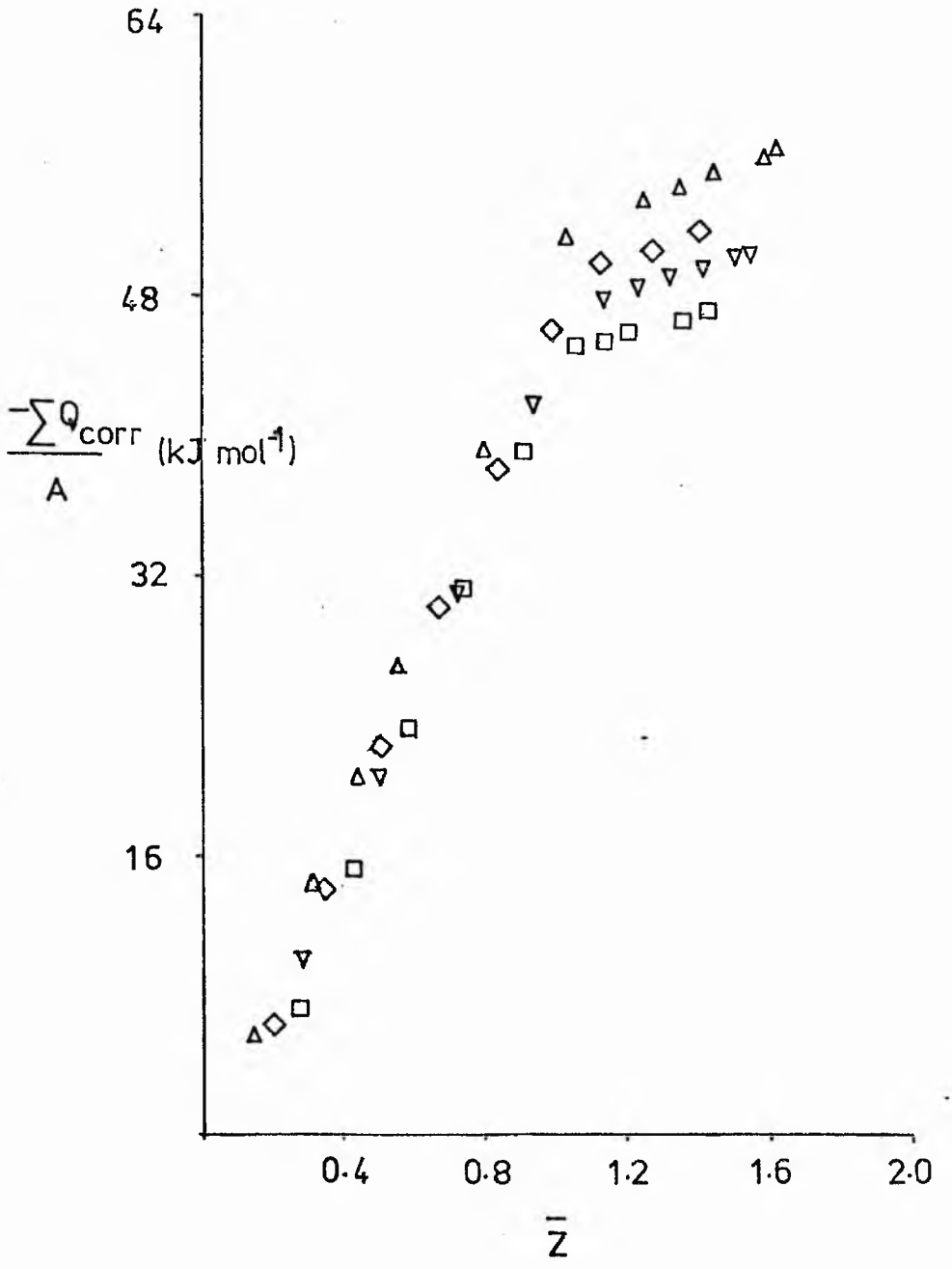


FIGURE 47 : ENTHALPIC CURVES FOR GLYCINATE PROTONATION

the carboxylate group being smaller and so less accurately measurable.

The enthalpy values determined from the program CALCO are -51.2 and -9.0 kJ mol^{-1} which may be assigned to the protonation of the amino and carboxylate groups respectively. The protonation of an amino group is an enthalpy dependent process whereas the protonation of a carboxylate group, essentially an electro-static phenomenon, is entropy dependent.

Other workers have reported values of ΔH_1 ranging from -41.9 [144] to -46.7 [121] kJ mol^{-1} and of $\Delta H_{1,2}$ ranging from -4.0 [145] to -6.0 [146] kJ mol^{-1}. However, these values invariably apply to lower ionic strengths than those reported here.

GLYCYLGLYCINATE PROTONATION

Experimental data are shown in appendix 3 - table 2 and enthalpic curves in figure 48.

The enthalpy values determined by CALCO are -48.7 and -5.6 kJ mol^{-1} which may be assigned to the protonation of the amino and carboxylate groups respectively. These results are in general agreement with those of other workers [123,147,148] considering the different conditions employed.

GLYCYLGLYCYLGLYCINATE PROTONATION

Experimental data are shown in appendix 3 - table 3 and enthalpic curves in figure 49.

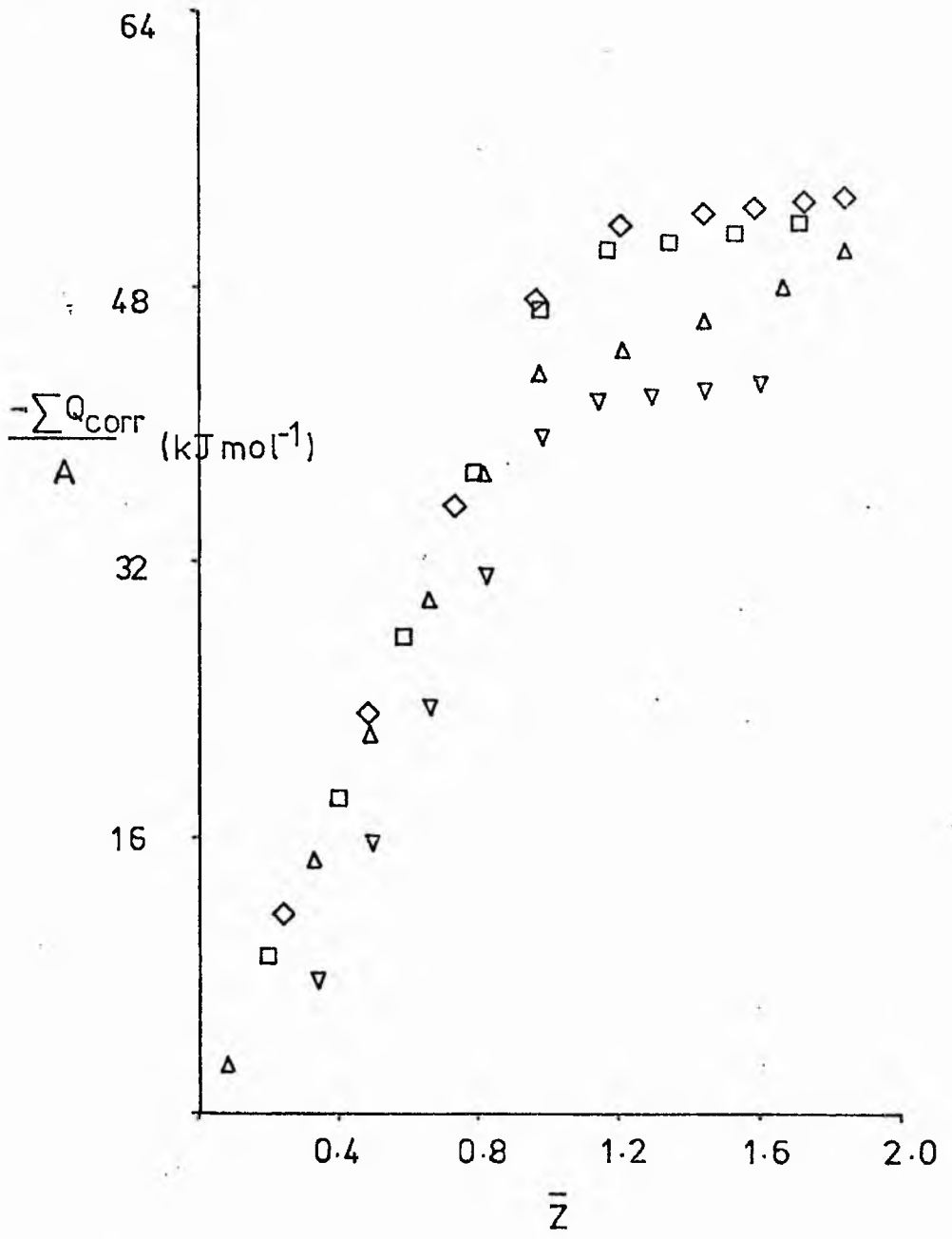


FIGURE 48 : ENTHALPIC CURVES FOR GLYCYLGLYCINATE PROTONATION

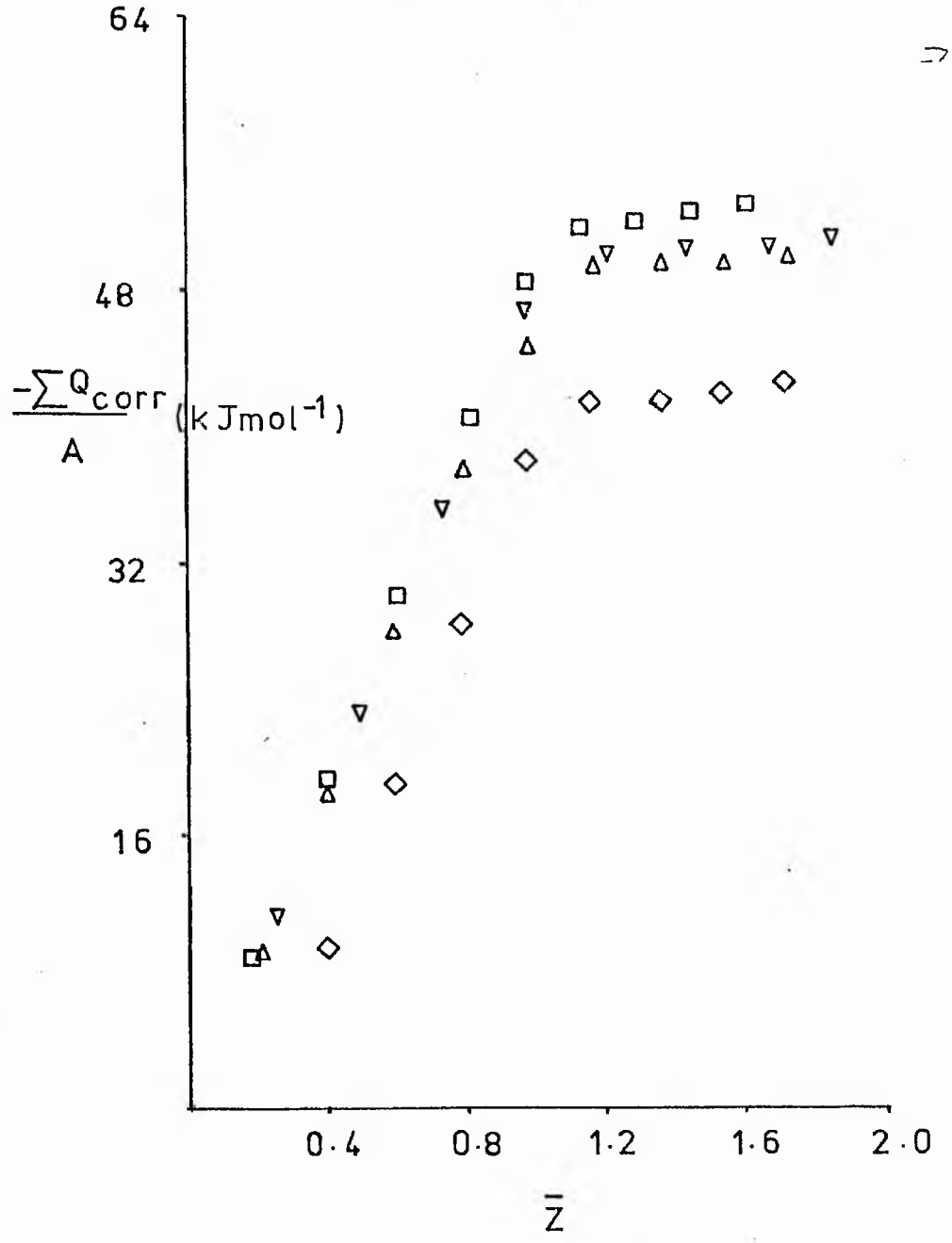


FIGURE 49 : ENTHALPIC CURVES FOR GLYCYLGLYCYLGLYCINATE PROTONATION

The enthalpy values determined by CALCO are -49.5 and -4.0 kJ mol^{-1} which may be assigned to the protonation of the amino and carboxylate groups respectively. Values reported in the literature for ΔH_1 are -23.0 [149] and -42.3 [147] kJ mol^{-1} and for $\Delta H_{1,2}$ -0.8 kJ mol^{-1} [147]. The results from reference 147 are in good agreement with those reported here considering that they were measured in anionic background of 0.1M $NaClO_4$.

GLUTATHIONATE PROTONATION

Experimental data are shown in appendix 3 - table 4 and enthalpic curves in figure 50.

The enthalpy values determined by CALCO are -37.1, -35.1, -1.8 and -4.6 kJ mol^{-1}. These are composite values which cannot be assigned to the protonation of individual groups since some concurrent protonation of the sulphydryl and amino groups and of the two carboxylate groups will be occurring. Vander Jagt *et al* [150] report the following calorimetrically determined values:- ΔH_1 -34.87, $\Delta H_{1,2}$ -31.65, $\Delta H_{2,3}$ -0.71 and $\Delta H_{3,4}$ -1.88 kJ mol^{-1} with which our values are in good agreement when one considers the difference in ionic strength.

A summary of the $\Delta H^{\ominus}$ values for ligand protonation, their standard deviations and the numbers of experimental observations is given in table 10.

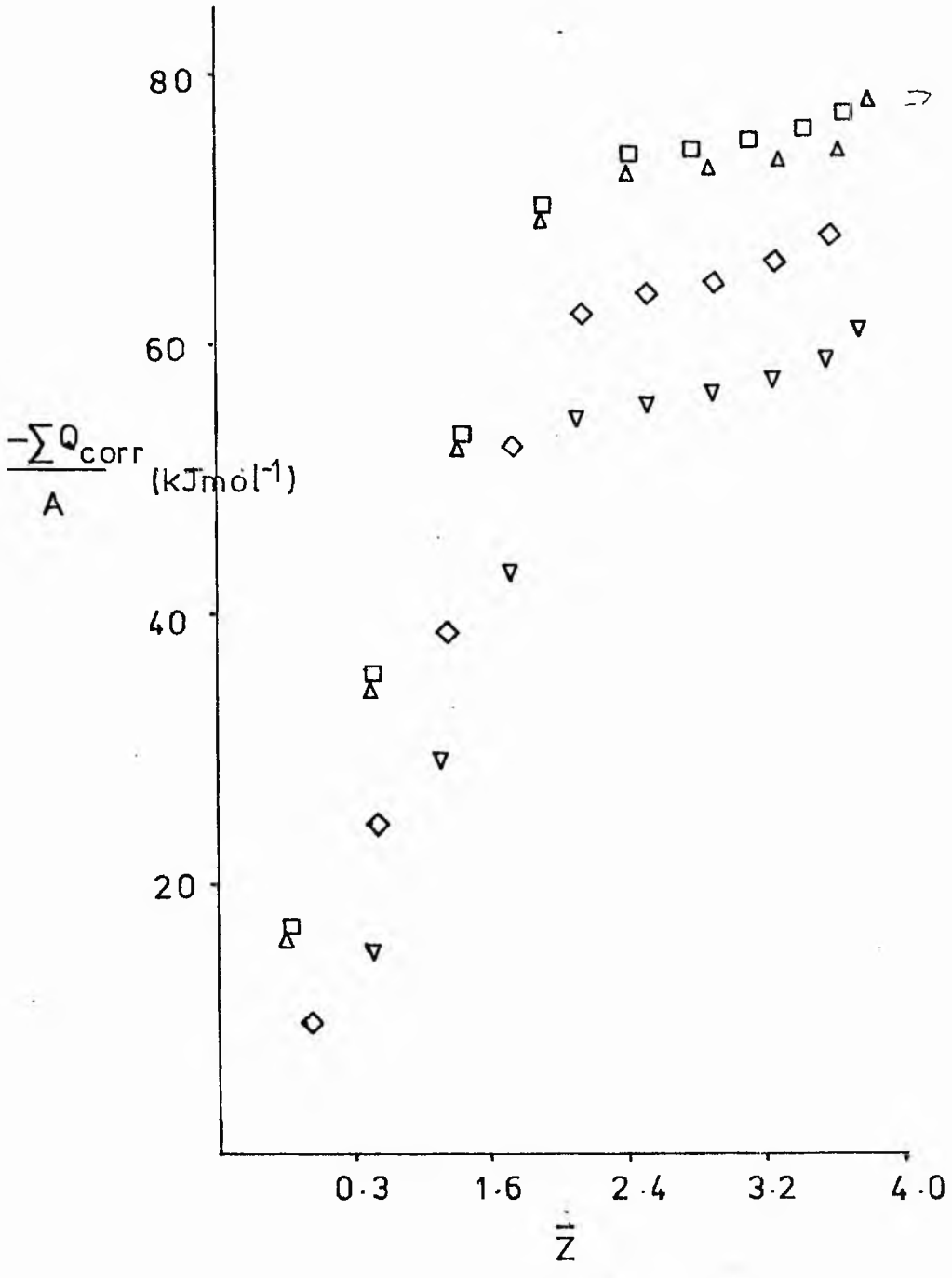


FIGURE 50 : ENTHALPIC CURVES FOR GLUTATHIONATE PROTONATION

TABLE 10

ENTHALPIES OF LIGAND ANION PROTONATION (kJ mol^{-1})

at 25°C, I = 3.00M(Na^+)ClO_4^-

	$\Delta H_1^\ominus$	$\Delta H_{1,2}^\ominus$	$\Delta H_{2,3}^\ominus$	$\Delta H_{3,4}^\ominus$	n*
Glycinate	-51.2 ± 1.3	-9.0 ± 0.4			40
Glycylglycinate	-48.7 ± 1.6	-5.6 ± 0.3			37
Glycylglycylglycinate	-49.5 ± 1.4	-4.0 ± 0.2			34
Glutathionate	-37.1 ± 1.0	-35.1 ± 1.4	-1.8 ± 1.9	-4.6 ± 3.4	36

* n = number of experimental observations

From these values of $\Delta H^\ominus$ and the log β values already measured, $\Delta G^\ominus$ and $\Delta S^\ominus$ were calculated as shown in Chapter 2. All three thermodynamic functions for the protonation of these four ligands are summarised in table 11.

TABLE 11

THERMODYNAMIC FUNCTIONS FOR LIGAND ANION PROTONATION ($\Delta G^{\ominus}$ and $\Delta H^{\ominus}$ in kJ mol^{-1} and $\Delta S^{\ominus}$ in J mol^{-1} K^{-1}) at 25°C, I = 3.00M(Na^{+})ClO_4^{-}

	Glycinate	Glycylglycinate	Glycylglycylglycinate	Glutathionate
$\Delta G_1^{\ominus}$	-57.49 ± 0.05	-48.88 ± 0.04	-49.10 ± 0.05	-56.41 ± 0.11
$\Delta G_{1,2}^{\ominus}$	-15.31 ± 0.11	-20.04 ± 0.08	-20.74 ± 0.17	-52.31 ± 0.20
$\Delta G_{2,3}^{\ominus}$				-21.80 ± 0.33
$\Delta G_{3,4}^{\ominus}$				-14.82 ± 0.43
$\Delta H_1^{\ominus}$	-51.2 ± 1.3	-48.7 ± 1.6	-49.5 ± 1.4	-37.1 ± 1.0
$\Delta H_{1,2}^{\ominus}$	-9.0 ± 0.4	-5.6 ± 0.3	-4.0 ± 0.2	-35.1 ± 1.4
$\Delta H_{2,3}^{\ominus}$				-1.8 ± 1.9
$\Delta H_{3,4}^{\ominus}$				-4.6 ± 3.4
$\Delta S_1^{\ominus}$	20.9 ± 3.1	0.8 ± 5.3	-1.3 ± 4.7	64.6 ± 3.7
$\Delta S_{1,2}^{\ominus}$	21.1 ± 1.7	48.3 ± 1.3	56.2 ± 0.6	57.9 ± 5.7
$\Delta S_{2,3}^{\ominus}$				67.1 ± 7.4
$\Delta S_{3,4}^{\ominus}$				49.7 ± 12.7

LEAD(II)-LIGAND INTERACTIONS

For the lead(II) complexing reactions calorimetry could not be used for the following reasons. For glycinate and its peptides, the maximum $\bar{Z}$ attainable before precipitation of lead hydroxides is about 1.0 and, even below this, the heat contribution from lead(II)-hydroxy species [36,37] is considerable thus making the net heat output low and the heats of the complexation reactions insignificant compared to the corrections which had to be applied. For the cysteinate and glutathionate-lead(II) systems, only very low concentrations of metal (~1mM) could be used in order to avoid precipitation of insoluble complexes and so again sufficient heat output to be accurately measurable could not be realised.

Thus, for the lead(II) complexing reactions, potentiometric studies have been carried out at 10, 25 and 40°C and the enthalpies of reaction calculated from the temperature variation of the formation constants.

Variation of Formation Constants with Temperature

Potentiometric work was carried out at 10, 25 and 40°C, I = 3.00M $(Na^+)ClO_4^-$ using the equipment and techniques described in Chapters 2 and 5. The enthalpies of ligand protonation, calorimetrically determined above and from reference 101, were used to calculate ligand protonation constants at 10 and 40°C. Similarly, formation constants for the four lead(II)-hydroxy species at 10 and 40°C were calculated from the enthalpies determined by Carell and Olin [37] (see table 12).

TABLE 12

ENTHALPIES OF LEAD(II)-HYDROXY COMPLEX FORMATION AT 25°C, I = 3.00M(Na^+)$\pounds ClO_4^-$ [37]

$\Delta H^{\ominus}_{O1-1}$	20.93 kJ mol^{-1}
$\Delta H^{\ominus}_{O4-4}$	84.01 kJ mol^{-1}
$\Delta H^{\ominus}_{O3-4}$	110.9 kJ mol^{-1}
$\Delta H^{\ominus}_{O6-8}$	207.0 kJ mol^{-1}

Having obtained log β values at different temperatures a plot of log β *versus* 1/T was drawn for each complex and the 'best' straight line drawn to give a gradient equal to

$$\frac{-\Delta H^{\ominus}}{2.303R}$$

from which $\Delta H^{\ominus}$ is calculated.

A summary of the measured enthalpies of complex formation, and the calculated free energies and entropies, is shown in table 18.

LEAD(II)-GLYCINATE

Experimental data are shown in appendix 3 - tables 5, 6 and 7. Our normal computational approach was used to give the log β values shown in table 13.

TABLE 13

LOG FORMATION CONSTANTS AT 10, 25 AND 40°C FOR LEAD(II)-GLYCINATE COMPLEXES

	log β_{pqr}			
	110	111	11-1	n
10°C	5.893 ± 0.034	12.212 ± 0.079	-2.071 ± 0.038	165
25°C	5.752 ± 0.045	11.880 ± 0.108	-1.886 ± 0.050	131
40°C	5.675 ± 0.027	11.772 ± 0.047	-1.781 ± 0.026	179

From these formation constants $\Delta H^{\ominus}$ values for the 110, 111 and 11-1 complexes were determined as -12.4, -25.1 and 16.5 kJ mol^{-1} respectively. No literature values are available for comparison but ΔH_{110} for zinc(II)-glycinate has been measured as -8.71 kJ mol^{-1} [121] and for cadmium(II)-glycinate as -8.87 kJ mol^{-1} [151] at considerably lower background ionic strengths.

LEAD(II)-GLYCYLGLYCINATE

Experimental data are shown in appendix 3 - tables 8, 9 and 10. The log β values determined from these results are shown in table 14.

TABLE 14

LOG FORMATION CONSTANTS AT 10, 25 AND 40°C FOR LEAD(II)-GLYCYLGLYCINATE COMPLEXES

	log β_{pqr}		
	110	111	n
10°C	4.005 ± 0.034	10.405 ± 0.059	139
25°C	3.824 ± 0.036	10.007 ± 0.063	99
40°C	3.782 ± 0.043	9.739 ± 0.077	156

From these formation constants $\Delta H^{\ominus}$ values for the 110 and 111 complexes were determined as -12.7 and -37.8 kJ mol^{-1} respectively.

LEAD(II)-GLYCYLGLYCYLGLYCINATE

Experimental data are shown in appendix 3 - tables 11, 12 and 13. The log β values determined from these results are shown in table 15.

TABLE 15

LOG FORMATION CONSTANTS AT 10, 25 AND 40°C FOR LEAD(II)-GLYCYLGLYCYLGLYCINATE COMPLEXES

	log β_{pqr}			
	110	111	11-1	n
10°C	4.143 ± 0.038	10.840 ± 0.046	-3.524 ± 0.061	96
25°C	3.967 ± 0.022	10.618 ± 0.021	-3.401 ± 0.024	103
40°C	3.879 ± 0.020	10.352 ± 0.020	-3.132 ± 0.017	97

From these formation constants $\Delta H^{\ominus}$ values for the 110, 111 and 11-1 complexes were determined as -15.0, -27.5 and 22.0 kJ mol^{-1} respectively.

LEAD(II)-CYSTEINATE

Experimental data are shown in appendix 3 - tables 14, 15 and 16. The log β values determined from these results are shown in table 16.

TABLE 16

LOG FORMATION CONSTANTS AT 10, 25 AND 40°C FOR LEAD(II)-CYSTEINATE COMPLEXES

	log β_{pqr}				
	110	111	210	211	n
10°C	13.579 ± 0.027	17.974 ± 0.069	17.84 ± 0.44	28.417 ± 0.065	96
25°C	13.207 ± 0.041	17.43 ± 0.11	18.23 ± 0.12	27.30 ± 0.12	147
40°C	12.828 ± 0.019	16.968 ± 0.047	17.547 ± 0.052	26.445 ± 0.058	179

From these formation constants ΔH^{e} values for the 110, 111 and 211 complexes were determined as -42.4, -56.9 and -111.8 kJ mol^{-1} respectively. For the 210 complex, which figure 19 shows to be present only at high pH, however, formation constants could not be obtained with sufficient accuracy to allow the enthalpy to be calculated.

LEAD(II)-GLUTATHIONATE

Experimental data are shown in appendix 3 - tables 17, 18 and 19. The log β values determined from these results are shown in table 17.

TABLE 17

LOG FORMATION CONSTANTS AT 10, 25 AND 40°C FOR LEAD(II)-GLUTATHIONATE COMPLEXES

	log β_{pqr}				
	110	111	211	212	n
10°C	10.769 ± 0.032	17.618 ± 0.015	24.453 ± 0.023	33.765 ± 0.020	147
25°C	9.913 ± 0.035	16.821 ± 0.017	23.400 ± 0.025	32.313 ± 0.022	128
40°C	9.583 ± 0.028	16.167 ± 0.013	22.665 ± 0.026	31.234 ± 0.019	113

From these formation constants $\Delta H^{\ominus}$ values for the 110, 111, 211 and 212 complexes were determined as -67.6, -82.2, -101.4 and -143.5 kJ mol^{-1} respectively. Again formation constants for the 210 complex, present only at high pH, could not be determined with sufficient accuracy to allow the enthalpy to be calculated.

The thermodynamic functions, which are summarised in table 18, are used to propose structures for the various complexes, as will be discussed in Chapter 8.

TABLE 18

THERMODYNAMIC FUNCTIONS FOR LEAD(II)-LIGAND ANION INTERACTIONS ($\Delta G^{\ominus}$ AND $\Delta H^{\ominus}$ in kJ mol^{-1} AND $\Delta S^{\ominus}$ in J mol^{-1} K^{-1}) AT 25°C, I = 3.00M(Na^{+})ClO_4^{-}

	Glycinate	Glycylglycinate	Glycylglycylglycinate	Cysteinate	Glutathionate
$\Delta G^{\ominus}_{110}$	-32.84 ± 0.25	-21.83 ± 0.20	-22.65 ± 0.12	-75.40 ± 0.23	-56.60 ± 0.20
$\Delta G^{\ominus}_{111}$	-67.82 ± 0.61	-57.13 ± 0.35	-60.62 ± 0.12	-99.53 ± 0.64	-96.03 ± 0.10
$\Delta G^{\ominus}_{11-1}$	10.76 ± 0.29		19.43 ± 0.13		
$\Delta G^{\ominus}_{211}$				-155.86 ± 0.69	-133.59 ± 0.14
$\Delta G^{\ominus}_{212}$					-184.48 ± 0.13
$\Delta H^{\ominus}_{110}$	-12.4 ± 3	-12.7 ± 5	-15.0 ± 1	-42.4 ± 2	-67.6 ± 5
$\Delta H^{\ominus}_{111}$	-25.1 ± 4	-37.8 ± 2	-27.5 ± 2	-56.9 ± 3	-82.2 ± 2
$\Delta H^{\ominus}_{11-1}$	16.5 ± 3		22.0 ± 4		
$\Delta H^{\ominus}_{211}$				-111.8 ± 4	-101.4 ± 3
$\Delta H^{\ominus}_{212}$					-143.5 ± 2
$\Delta S^{\ominus}_{110}$	68.6 ± 11	30.5 ± 17	25.5 ± 4	110.6 ± 7	-36.8 ± 17
$\Delta S^{\ominus}_{111}$	143.2 ± 15	34.3 ± 8	111.0 ± 7	142.9 ± 12	46.4 ± 7
$\Delta S^{\ominus}_{11-1}$	19.2 ± 11		8.7 ± 14		
$\Delta S^{\ominus}_{211}$				147.8 ± 16	107.9 ± 10
$\Delta S^{\ominus}_{212}$					137.6 ± 7

CHAPTER 7

RESULTS - BOVINE SERUM ALBUMIN

CONTENTS

CHAPTER 7

RESULTS - BOVINE SERUM ALBUMIN

The amino acid sequence for bovine serum albumin(BSA) shown in reference 95 gives 578 of the 581 residues and leads to a molecular weight of approximately 66,000 and so this is the value used in subsequent work.

Potentiometry

It was found that BSA solutions precipitated around pH 4 to 5 in perchlorate or nitrate, even at background concentrations as low as 0.150M, and in chloride at concentrations of greater than 1.0M. Thus the ionic background chosen for the potentiometric work was 0.150M $(Na^+)Cl^-$. Since this change to plasma ionic strength was necessitated, it was decided also to use the biological temperature, 37^oC.

A glass electrode and a calomel reference electrode were used, as in Chapter 5, but, of course, these had to be recalibrated under the new experimental conditions. Unfortunately, a relevant value of pw_k was not available and so this was calculated from the calibration

line. The theoretical slope of the calibration line at 37°C is -61.544mV(-logh)$^{-1}$ and this is also assumed to be the slope for the experimental line above pH 7. Thus, by applying a least-squares analysis to several calibration lines, pw_k is calculated as -13.30 ± 0.04 and this value is used in subsequent work.

BOVINE SERUM ALBUMIN PROTONATION

Experimental data are shown in appendix 4 - table 1 and in figure 51.

The ZPLOT program had to be slightly modified [86] in order to be able to deal with a ligand with such a large number of protonatable sites.

Brown's analysis [95] shows 38 aspartate residues and 57 glutamate residues as well as 5 residues which may be aspartate or asparaginate and 7 residues which may be glutamate or glutaminate. This means that the number of protons which the BSA molecule can release, within the pH range studied, is between 95 and 107. Österberg has assumed [152] 99 carboxylate groups on the BSA molecule which agrees with Brown's analysis and so this value for the number of dissociable protons has been used in this work.

The formation curves in figure 51 show maximum $\bar{Z}$s of 189 ± 2. From the observed changes in curve slope it is possible to estimate the numbers of the main types of electron donor groups present.

(i) At ph 5.5, $\bar{Z}$ = 93 and so the number of carboxylate groups present is 189 - 93 = 96 ± 3 which agrees with the number found by Österberg [152] and Brown [95].

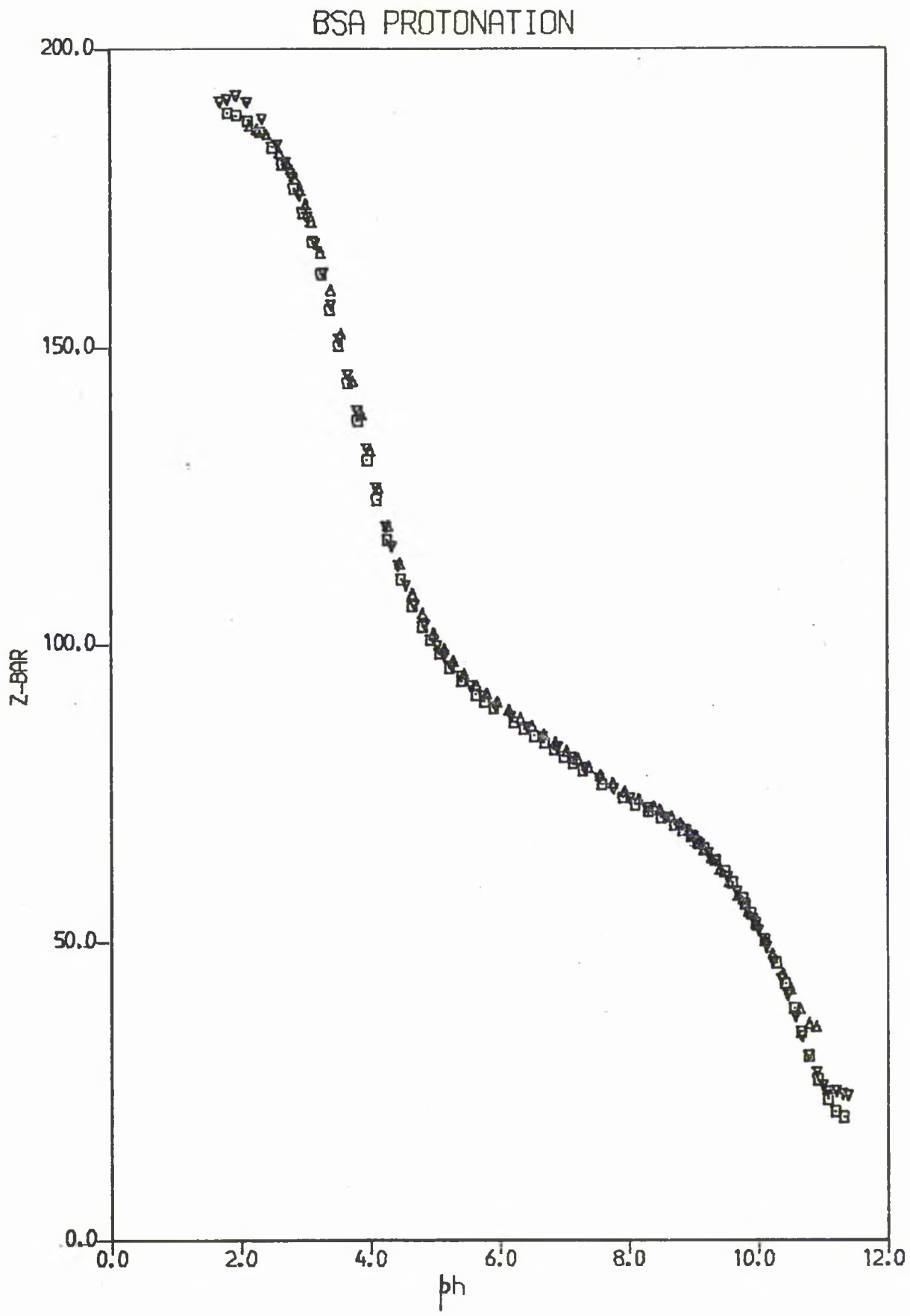


FIGURE 51 : ZPLOT OF DATA FROM APPENDIX 4 - TABLE 1

(ii) At ph 8.0, $\bar{Z}$ = 75 and so the number of imidazole groups present is 93 - 75 = 18 ± 2. This again agrees well with other analyses which find 17 histidinate residues [95,153-155].

(iii) This leaves 75 other protonatable sites unaccounted for which must include lysinate, tyrosinate and cysteinate side chains. Brown's[95] analysis gives 59 lysinate, 19 tyrosinate and 1 cysteinate residues, making a total of 79.

LEAD(II)-BOVINE SERUM ALBUMIN INTERACTION

Several titrations were carried out in which a solution of lead(II), BSA and acid was titrated with alkali, with varying ratios of metal to protein being used. For high ratios of lead(II) to BSA precipitation of insoluble complexes occurs around ph 4 but these redissolve at about ph 9. The insolubility of lead(II)-BSA complexes has been previously reported [152].

Experimental data are shown in appendix 4 - table 2. Since protonation constants are not available for each site on the ligand, it was not possible to compute a $\bar{Z}$ *versus* pa curve. However, figure 52 shows the effect that the presence of lead(II) has on the BSA protonation curves. As can be seen, from ph 5 upwards the presence of lead(II) tends to move the curves to lower $\bar{Z}$ at any given ph i.e. protons are apparently being released from BSA by the metal. The extent of the shift in the curves depends on the ratio of lead(II) to BSA.

Table 19 shows the number of protons released for each titration at biological ph (7.4) and at ph 10.0.

It appears that the number of protons released is approximately proportional to B/A and that at ph 10.0 twice as many are released as at ph 7.4. It is possible that at these phs the formation of lead(II)-hydroxy species, as well as lead(II)-BSA species, is causing proton release.

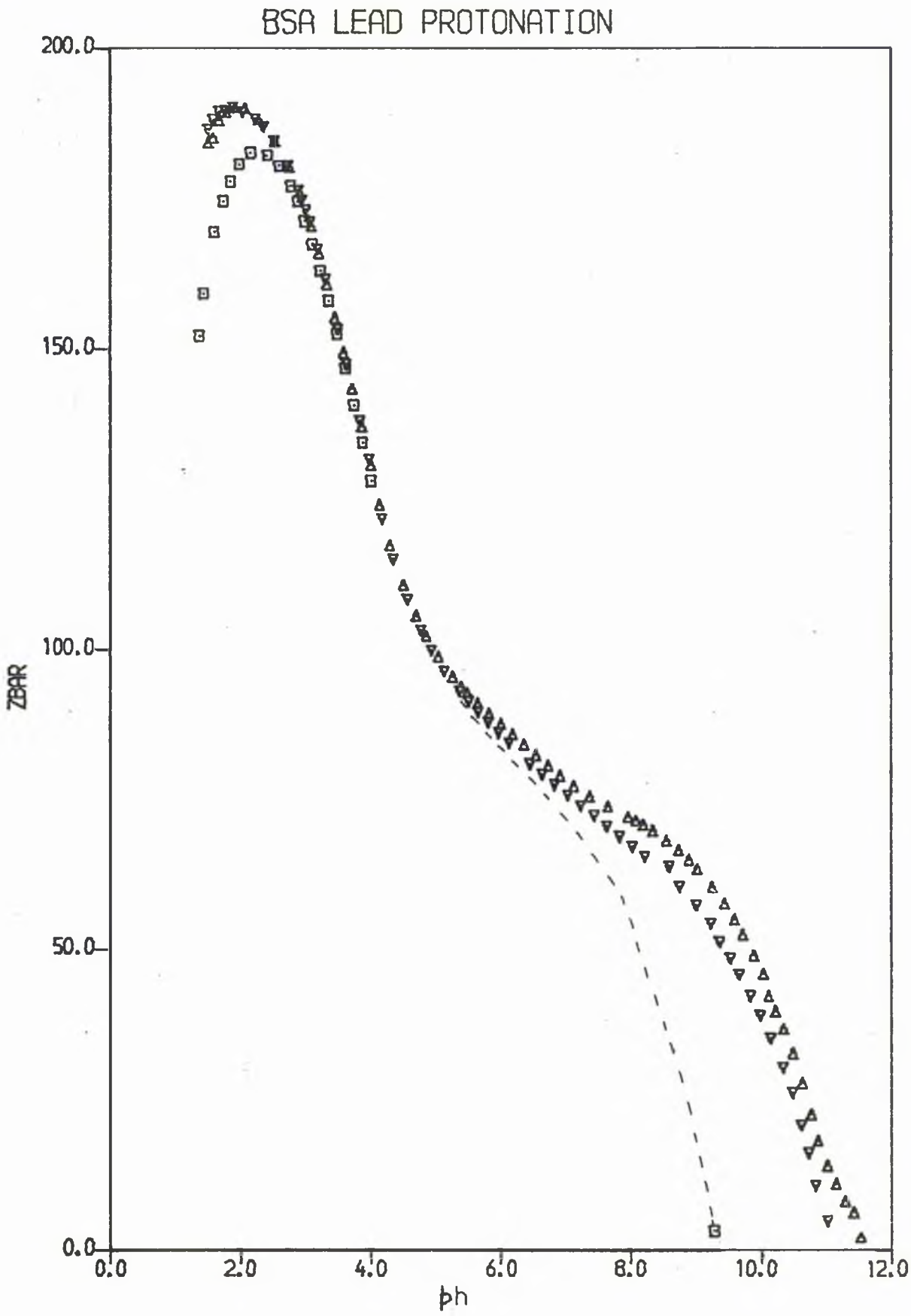


FIGURE 52 : ZPLOT OF DATA FROM APPENDIX 4 - TABLE 2

TABLE 19

THE APPARENT DISPLACEMENT OF PROTONS FROM BSA BY LEAD(II)

ph	B : A	$\bar{Z}$	$\Delta\bar{Z}$
7.4	0 : 1	78.7	0
	5 : 1	75.3	3
	9 : 1	72.5	6
	45 : 1	precipitate present	
10.0	0 : 1	52.3	0
	5 : 1	46.6	6
	9 : 1	38.5	14
	45 : 1	-14.6	66

COPPER(II)-BOVINE SERUM ALBUMIN INTERACTION

Experimental data are shown in appendix 4 - table 3 and ligand protonation curves drawn from these results in figure 53. Again some precipitation of metal-BSA complexes occurred around the centre of the ph range.

These curves are shifted from the true ligand protonation curve even at very low ph (~2) and the shift is much more marked than in the lead(II) case, although the extent of the shift still depends on the ratio of copper(II) to BSA. Table 20 shows the number of protons released for each titration at ph 4.0, 7.4 and 10.0.

As can be seen, many more protons are released by copper(II) than by lead(II) and at lower ph and so it can be stated that copper(II) complexes to BSA more effectively than lead(II). This, obviously, is due to the presence of the specific copper(II) binding site on albumin whereas lead(II) is believed to bind only to carboxylate groups [152]. Zinc(II) was found [86] to have an intermediate effect (see figure 54).

Copper(II)-Bovine Serum Albumin - Drug Interactions

ULTRA-VIOLET/VISIBLE SPECTROSCOPY

Aqueous solution

Preliminary investigations of bovine serum albumin (0.1mM), in 0.150M NaCl at pH 7.4, produced ultra-violet absorption peaks at

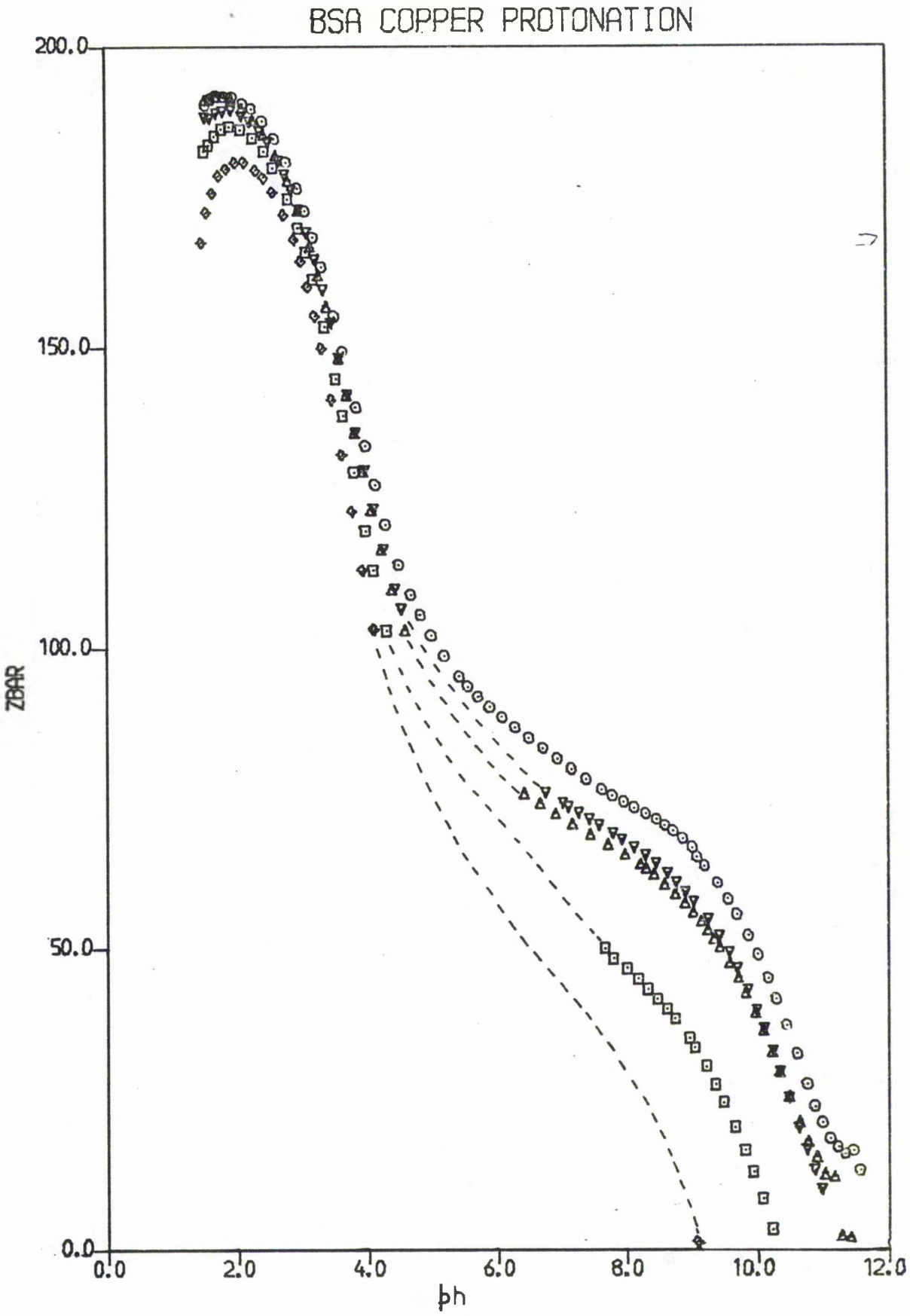


FIGURE 53 : ZPLOT OF DATA FROM APPENDIX 4 - TABLE 3

TABLE 20

THE APPARENT DISPLACEMENT OF PROTONS FROM BSA BY COPPER(II)

ph	B : A	$\bar{Z}$	$\bar{Z}$
4.0	0 : 1	131.4	0
	1 : 1	133.2	-2
	4 : 1	127.4	4
	9 : 1	118.0	13
	22 : 1	109.6	22
7.4	0 : 1	78.7	0
	1 : 1	78.0	1
	4 : 1	70.5	8
	9 : 1	~52	~26.
	22 : 1	precipitate present	
10.0	0 : 1	52.3	0
	1 : 1	49.0	3
	4 : 1	39.1	13
	9 : 1	10.6	42
	22 : 1	-29.7	82

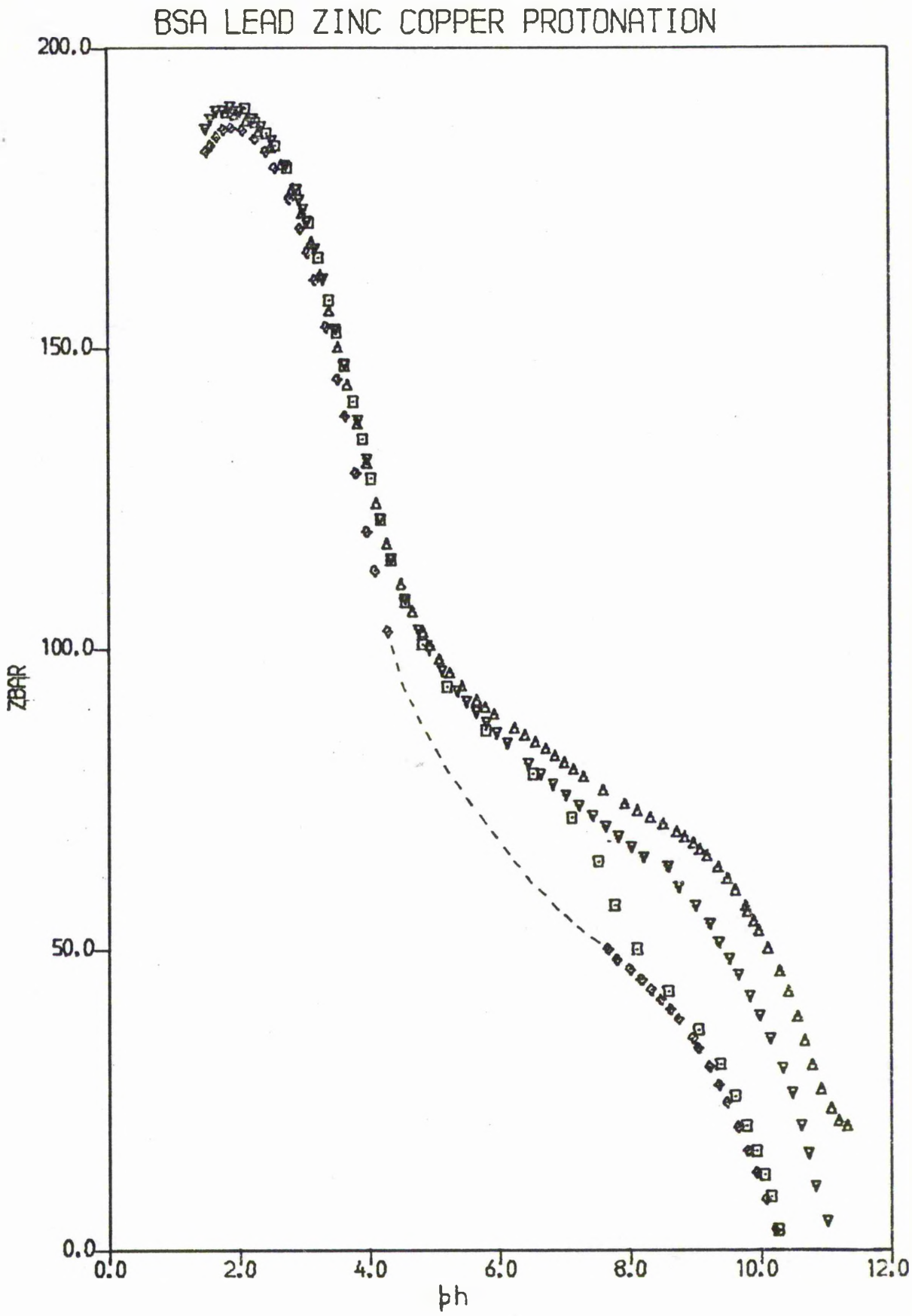


FIGURE 54 : THE EFFECT OF LEAD(II), ZINC(II) AND COPPER(II) ON BSA PROTONATION

△ = BSA/H^+ ; ▽ = Pb : BSA = 9:1

⊡ = Zn : BSA = 20:1 ; ◇ = Cu : BSA = 9:1

205(strong) and 278(weak) nm. One of the more water soluble of the drugs shown in figure 2, from the middle of the potency order, (Fenoprofen) was studied under similar conditions and exhibited peaks at 209 and 270nm, the latter having shoulders at 263 and 278nm.

When copper(II) chloride (0.1mM) was introduced into these solutions there was negligible effect on the BSA spectrum and an immediate precipitate with Fenoprofen. Lower concentrations, although producing soluble systems, did not exhibit any copper influence upon absorption peak positions. Higher concentrations (2mM Cu) did show the influence of copper(II) on BSA (a shoulder appeared on the 278nm peak at 357nm) but exceeded the solubility of the Fenoprofen. Thus, in order to investigate the influence of the drugs on the binding of copper(II) to BSA, more concentrated solutions are necessary and so a change of solvent is indicated.

Ethanolic solution

The conditions chosen were 90% ethanol/water at pH 2.4 (BSA is insoluble in ethanol above pH 3). These permitted higher concentrations of drugs to be used (up to 10mM) and eliminated interference from direct copper(II)-drug interactions although it did introduce the possibility that the BSA would be denatured by the alcohol.

Under these conditions it is not possible to use the BSA ultra-violet absorption peaks because they are swamped by drug and copper peaks but studies of the visible spectrum are feasible. Such investigations show that when BSA (0.200mM) was introduced into a copper(II) solution (11.17mM) the wavelength of the absorption peak

maximum decreased (780 to 759nm) and the absorbance increased (0.770 to 1.215). Fenoprofen (10.00mM), on the other hand, does not affect the absorption of the copper(II) solution but does influence copper(II)-BSA solutions by moving the peak back towards the free copper(II) peak (i.e. to 764nm). Thus it would appear that Fenoprofen liberates copper(II) from BSA.

Similar investigations were carried out with the other anti-arthritic drugs as well as with salicylate and histidinate.

Table 21 shows the copper(II)-drug interactions in the absence of bovine serum albumin and it is clear from the shifts in λ_{max} that only salicylic acid and histidine are appreciably complexed to copper(II) at pH 2.4. At pH 3.9, however, it is seen that adding the drugs to a copper(II) solution lowers the λ_{max} and increases the absorbance (Phenylbutazone is a slight exception in terms of λ_{max}).

The absorbance of a solution of copper(II) ions, at 780nm say, is given by $abs_{780} = \epsilon'_{780}\, d\, [\,]$ where ϵ'_{780} = the absorption coefficient for copper(II) ions at 780nm, d = path length and [ ] = concentration of copper(II) ions. For example, at pH 2.4 for a copper(II) chloride solution ϵ'_{780} can be calculated as 0.0171 l $mmol^{-1}$ cm^{-1} and this value does not vary significantly between pH 2.1 and pH 4.0.

When a drug is added to this solution, then

$$abs_{780} = \epsilon'_{780}\, d([\,] - x) + \epsilon''_{780}\, d\, x$$

where ϵ''_{780} = the absorption coefficient for the copper-drug complex and x = the concentration of copper(II) transferred from ions in solution into the drug complexed form.

TABLE 21

SPECTRAL RESULTS FOR COPPER(II)-DRUG INTERACTIONS

	Concentrations used (mM)			Spectral data			
pH	Copper(II)	Drug		λ_{max} (nm)	abs_{max}	abs_{780}	abs_{700}
2.4	11.17			780	0.770	0.770	0.440
	11.17	Fenoprofen	10.00	780	0.815	0.815	0.480
	11.17	Indomethacin	10.00	780	0.820	0.820	0.485
	11.17	Naproxen	10.00	780	0.820	0.820	0.500
	11.17	Ketoprofen	10.00	779	0.820	0.820	0.495
	11.17	Phenylbutazone	10.00	780	0.790	0.790	0.470
	11.17	Aspirin	10.00	779	0.825	0.825	0.505
	11.17	salicylic acid	10.00	776	0.925	0.920	0.590
	11.17	histidine	10.00	692	1.480	1.190	1.475
	11.17	histidine	1.000	776	0.840	0.835	0.590
	11.17	histidine	0.200	778	0.795	0.795	0.490
3.9	11.17			780	0.770	0.770	0.440
	11.17	Fenoprofen	10.00	766	1.225	1.200	0.925
	11.17	Indomethacin	10.00	769	1.225	1.210	0.905
	11.17	Naproxen	10.00	768	1.180	1.165	0.865
	11.17	Ketoprofen	10.00	769	1.170	1.160	0.850
	11.17	Phenylbutazone	10.00	782	0.810	0.810	0.480
	11.17	Aspirin	10.00	772	1.145	1.135	0.695
	11.17	salicylic acid	10.00	765	1.360	1.330	1.010
	11.17	histidine	1.000	774	0.875	0.870	0.620
	11.17	histidine	0.200	779	0.810	0.810	0.525

Thus from table 21 it would appear that $\epsilon''_{780} > \epsilon'_{780}$. Provided that any variation in ϵ''_{780} from drug to drug is not large, then the copper(II)-drug complexing order is seen to be

histidine >> salicylic acid > Indomethacin ~ Fenoprofen > Naproxen ~ Ketoprofen > Aspirin > Phenylbutazone.

Table 22 indicates the effect of BSA on these results, data being recorded at pH 2.4 in order to avoid the above copper(II)-drug interactions noted at pH 3.9.

TABLE 22

SPECTRAL RESULTS FOR COPPER(II)-BSA-DRUG INTERACTIONS AT pH 2.4

Concentrations used (mM)				Spectral data			
Copper(II)	BSA	Drug		λ_{max} (nm)	abs_{max}	abs_{780}	abs_{700}
11.17				780	0.770	0.770	0.440
11.17	0.200			759	1.215	1.175	1.040
11.17	0.200	Fenoprofen	(10.00)	764	1.160	1.135	0.955
11.17	0.200	Indomethacin	(10.00)	764	1.240	1.205	1.030
11.17	0.200	Naproxen	(10.00)	761	1.240	1.210	1.050
11.17	0.200	Ketoprofen	(10.00)	760	1.210	1.175	1.025
11.17	0.200	Phenylbutazone	(10.00)	764	1.155	1.140	0.950
11.17	0.200	Aspirin	(10.00)	762	1.240	1.205	1.040

Clearly, there is extensive copper(II)-BSA complexing even at pH 2.4, since, when BSA is added to the copper(II) solution, λ_{max} changes from 780 to 759nm. Further, at all wavelengths $\epsilon^{Cu\text{-}BSA} > \epsilon'$. Since no copper(II)-drug interactions occur at this pH the absorbance, and peak position, changes must arise from copper ions being transferred between BSA and solution. So, a peak shift towards higher wavelengths and a decrease in absorbance indicates a release of albumin bound copper(II), not through direct copper(II)-drug complexing, but, rather, through the drug acting remotely on the BSA molecule. An approximate remote copper(II) releasing order would thus read

Fenoprofen ~ Phenylbutazone > Ketoprofen > Indomethacin ~ Aspirin ~ Naproxen

Histidine and salicylic acid, on the other hand, are capable of direct competition for the copper(II) ion at pH 2.4 as can be seen from the data in table 23.

Thus, we see that salicylic acid and especially histidine are much more effective at releasing copper(II) from BSA than the remote copper(II) releasing drugs.

Finally we considered the effect of a remote copper(II) releasing drug (Fenoprofen) and a direct copper(II) complexing ligand (histidine) acting in unison, see table 24.

Surprisingly, it appears that the presence of histidine prevents Fenoprofen from having its remote copper(II) releasing effect. However, this may be accounted for since any copper(II) released by Fenoprofen

TABLE 23

SPECTRAL RESULTS FOR COPPER(II)-BSA-HISTIDINE AND -SALICYLIC ACID INTERACTIONS AT pH 2.4

Concentrations used (mM)				Spectral data			
Copper(II)	BSA	Drug		λ_{max} (nm)	abs_{max}	abs_{780}	abs_{700}
11.17				780	0.770	0.770	0.440
11.17	0.200			759	1.215	1.175	1.040
11.17		Salicylic acid	(10.00)	776	0.925	0.920	0.590
11.17	0.200	Salicylic acid	(10.00)	765	1.220	1.200	0.975
11.17		histidine	(10.00)	692	1.480	1.190	1.475
11.17	0.200	histidine	(10.00)	690	1.675	1.320	1.665
11.17		histidine	(1.000)	776	0.840	0.835	0.590
11.17	0.200	histidine	(1.000)	760	1.235	1.200	1.075

TABLE 24

SPECTRAL RESULTS FOR COPPER(II)-BSA-FENOPROFEN-HISTIDINE INTERACTIONS AT pH 2.4

Concentrations used (mM)				Spectral data			
Copper(II)	BSA	Histidine	Fenoprofen	λ_{max} (nm)	abs_{max}	abs_{780}	abs_{700}
11.17				780	0.770	0.770	0.440
11.17	0.200			759	1.215	1.175	1.040
11.17		1.000	10.00	775	0.910	0.905	0.64
11.17	0.200	1.000	10.00	758	1.210	1.170	1.040

would not be free in solution but would be bound to histidine thus lowering λ_{max} as can be seen from table 23.

There is no guarantee that these spectral investigations, conducted in conditions far removed from biological fluids and pH values, reflect the reactions occurring *in vivo* and so the observations were checked using the molecular filtration technique at pH 7.4.

MOLECULAR FILTRATION

An equimolar aqueous solution of copper(II) chloride and drug (0.1475mM) is adjusted to pH 7.4 and filtered at a flow rate of 1 ml min^{-1}, each 1ml fraction being collected, diluted to 10ml and analysed for

copper by atomic absorption spectrophotometry. The process of filtration disturbs the equilibrium in the solution under study and so only the first fraction is considered. Measurements at different pH values show that as the pH is raised more copper is retained as precipitated copper hydroxides. Thus correction factors to account for this have to be applied to the drug binding data.

Next a solution of copper(II), drug and BSA is filtered at pH 7.4 and so the ability of the drug to release copper(II) from BSA is quantified. The experimental data is summarised in table 25.

From these results, the ability of the drugs to bind copper(II) at pH 7.4 is in the order

histidine >> threonine >> salicylic acid ~ Naproxen
> Indomethacin > Fenoprofen ~ Ketoprofen ~ Phenylbutazone

Table 26 summarises the percentages of copper(II) bound to BSA in the presence of the drugs studied.

Thus the order of ability of the drugs to release copper(II) from bovine serum albumin at pH 7.4 is

histidine >> Indomethacin > Phenylbutazone > threonine
~ Ketoprofen ~ Fenoprofen > Naproxen ~ salicylic acid > Aspirin

This order, of course, does not discriminate between direct and remote release of copper(II) ions.

A comparison of the results of the spectral and molecular filtration studies with the drug effectiveness order will be found in Chapter 8.

TABLE 25

MOLECULAR FILTRATION DATA FOR COPPER(II)-BSA-DRUG INTERACTIONS

(x = 0.1475mM)

		Concentrations used			% copper(II)		
	pH	Copper(II)	BSA	Drug(s)	in filtrate	not bound to BSA	bound to BSA
Aspirin	4.0	x		x	77		
	6.0	x		x	45		
	7.4	x		x	41-47		
	4.0	x	x		36	47	53
	6.0	x	x		<1		>99
	7.4	x	x		<1		>99
	4.0	x	x	4x	35	45	55
	7.4	x	x	4x	<1		>99
histidine	7.4	x		2x	93	100	0
	7.4	x	x	2x	33	35	65
threonine	7.4	x		2x	78	100	0
	7.4	x	x	2x	5	6	94
histidine and	7.4	x		x+x	86	100	0
threonine	7.4	x	x	x+x	16	19	81
salicylic acid	7.4	x		2x	44	100	0
	7.4	x	x	2x	1.1	2.5	97.5

Table 25 continued

Fenoprofen	7.4	x		2x	26	100	0
	7.4	x	x	2x	1.4	5.4	95
Indomethacin	7.4	x		2x	33	100	0
	7.4	x	x	2x	4.2	13	87
histidine and	7.4	x		2x+2x	93	100	0
Indomethacin	7.4	x	x	2x+2x	38	41	59
Naproxen	7.4	x		2x	43	100	0
	7.4	x	x	2x	1.1	2.6	97
Ketoprofen	7.4	x		2x	24	100	0
	7.4	x	x	2x	1.1	4.6	95
Phenylbutazone	7.4	x		2x	22	100	0
	7.4	x	x	2x	2.2	10	90

TABLE 26

RESULTS FROM THE MOLECULAR FILTRATION STUDY AT pH 7.4

Ratio of Concentrations			% copper(II)
Cu :	BSA :	Other components	bound to BSA
1	1	0	>99
1	1	4 Aspirin	>99
1	1	2 histidine	65 ± 2
1	1	2 threonine	94 ± 1
1	1	1 histidine : 1 threonine	81 ± 1
1	1	2 salicylic acid	97.5 ± 1
1	1	2 Fenoprofen	95 ± 1
1	1	2 Indomethacin	87 ± 1
1	1	2 histidine : 2 Indomethacin	59 ± 2
1	1	2 Naproxen	97 ± 1
1	1	2 Ketoprofen	95 ± 1
1	1	2 Phenylbutazone	90 ± 1

CHAPTER 8

DISCUSSION

CONTENTS

CHAPTER 8

DISCUSSION

For the purposes of discussion the work reported here will be divided into its three major sections and the 'Challenge' project results will be discussed separately in Chapter 9.

Computer Models of Relevance to the Treatment of Lead Poisoning

The COMPLOT program and the formation constants whose determination has been described in this thesis have been used to construct model systems in order to evaluate the effectiveness of current and proposed treatments for lead poisoning.

Plumbism can be diagnosed at blood lead concentrations of 2.89μM but, regrettably, levels considerably higher than this are common. Normal levels of other species present in blood are shown in table 27.

Firstly, we considered the complexing of a representative amino acid, serine, to the metals shown in table 27 in the presence of lead(II) concentrations of 0, 10 and 50μM. The formation constants used are listed in table 28.

TABLE 27

Total concentrations of species used in blood plasma models [38]

	μM		μM
Asparagine	43.9	Tryptophan	58.2
Aspartic acid	2.2	Mn(II)	0.73
Cysteine	108.0	Fe(II)	23.3
Glutamine	62.3	Co(II)	1.72
Histidine	81.5	Cu(II)	18.25
Phenylalanine	55.3	Zn(II)	45.88
Serine	106.5		

TABLE 28

Log formation constants for serinate complexes [73]

	pqr	$\log \beta_{pqr}$		pqr	$\log \beta_{pqr}$
H	101	9.574	Cu(II)	110	8.950
	102	12.133		210	16.230
Mn(II)	110	2.893	Zn(II)	110	4.898
	210	4.791		210	9.279
Fe(II)	110	4.299		310	11.909
	210	7.377	Pb(II)*	110	4.948
	310	10.299		210	8.145
Co(II)	110	4.584		310	9.873
	210	8.568			
	310	11.554			

* this work

These models show that the lead(II) complexes are formed almost entirely at the expense of the zinc(II) complexes.

Now all eight amino acids are considered complexing with zinc(II) and lead(II), the formation constants used being shown in table 29.

These calculations show that it is predominantly the zinc(II)-cysteinate complexes which are robbed by lead(II) to give high concentrations of lead(II)-cysteinate species. The zinc(II) which is thus released is now free to complex with the other amino acids and, indeed, increased concentrations of zinc(II) complexes, such as zinc(II)-histidinate, are found. So we must realise that increased concentrations of an essential metal ion's complexes may be an indication of the presence of a polluting metal ion.

We then consider the effect of the ligands D-penicillaminate(D-pen) and ethylenediaminetetraacetate(edta), which are currently used to treat lead poisoning, on the lead(II), zinc(II), cysteinate, histidinate complexing system. The formation constants used in these models are shown in table 30.

In these models we find a high concentration of lead(II)-D-pen complexes, as we require, but also, unfortunately, a high concentration of zinc(II)-D-pen complexes. The concentrations of the lead(II)-cysteinate complexes decrease as the ligand is replaced by the drug. This allows the zinc(II) to return to the cysteinate complexed forms again and so these concentrations increase while the levels of the other zinc(II) complexes fall because of the competition from the drug.

A very similar situation arises in the case of edta.

TABLE 29

Log formation constants for amino acid anion-proton, -lead(II) and -zinc(II) complexes

	pqr	log β_{pqr}		pqr	log β_{pqr}
Asparaginate H[101]	101	9.303	Histidine H[158]	101	9.630
	102	11.888		102	16.600
Pb(II)*	110	4.902		103	18.882
	210	7.802	Pb(II)*	110	6.901
	310	8.841		210	9.797
Zn(II)[156]	110	5.070	Zn(II)[158]	110	7.068
	210	9.426		210	12.741
	310	12.300	Phenylalaninate H[101]	101	9.610
Aspartate H[101]	101	10.008		102	12.364
	102	14.074	Pb(II)*	110	4.314
	103	16.419		210	8.045
Pb(II)*	110	6.878	Zn(II)[157]	110	4.863
	210	10.014		210	9.598
	111	12.695	Serinate H[73]	101	9.574
	211	19.181		102	12.133
	212	24.985	Pb(II)*	110	4.948
	112	16.167		210	8.145
Zn(II)[157]	110	6.379		310	9.873
	210	11.539	Zn(II)[73]	110	4.898
	111	11.927		210	9.279

Table 29 continued

	pqr	log β_{pqr}
Cysteinate H[101]	101	10.709
	102	19.493
	103	21.933
Pb(II)*	110	13.213
	210	18.571
	111	17.347
	211	27.476
Zn(II)*	210	19.395
	211	25.856
	212	31.879
	430	46.247
	431	52.503
Glutaminate H[73]	101	9.640
	102	12.361
Pb(II)*	110	4.132
	210	7.083
	310	9.805
Zn(II)[73]	110	4.826
	210	9.165
	310	11.843
Tryptophanate H[158]	101	9.923
	102	12.676
Pb(II)*	110	4.517
	210	9.580
Zn(II)[158]	110	5.013
	210	9.779
	310	13.498

* this work

TABLE 30

Log formation constants for drug - proton, - lead(II) and zinc(II) complexes

	pqr	log β_{pqr}		pqr	log β_{pqr}
D-penicillaminate			Ethylenediaminetetraacetate		
H[30]	101	11.010	H*	101	9.060
	102	19.612		102	16.100
	103	22.044		103	18.680
Pb(II)*	110	14.321		104	20.953
	210	19.049	Pb(II)*	110	15.186
	111	17.723		111	18.010
	211	27.978	Zn(II)*	110	14.873
Zn(II)[30]	210	20.521		111	17.965
	211	26.794			
	212	32.724			
	430	47.582			
	431	53.826			
	21-1	8.563			

* this work

These results show that the drugs are insufficiently selective and that the *in vivo* cysteinate seriously challenges the therapeutic system.

For effective treatment of lead poisoning we require a ligand which will be more selective than D-pen or edta, which is, preferably, naturally occurring and so less likely to be toxic, and which is easily degraded and excreted by the body. Glutathionate, blood levels of which have been shown [159] to be lowered during lead poisoning, or indeed cysteinate itself, fulfills these criteria and so further models were computed considering these as the added therapeuticals. The formation constants used were those determined in this work.

The four ligands edta, D-pen, cysteinate and glutathionate were compared using the model system; [Zn(II)] = 45.88μM; [Pb(II)] = 10.0μM; and [ligand] = 20.0μM at pH 7.4, see table 31(a).

COMPLOT models were also computed over a range of lead(II) concentrations and glutathionate to lead(II) ratios, see table 31(b).

Thus the most effective ratio of glutathionate : lead(II) is 2 : 1, the higher ratios removing more of the essential zinc(II). However, no matter which ligand is used some zinc(II) will be removed and so will have to be replaced by zinc supplementation. To be successful the administered glutathionate would have to be kept in the reduced form, perhaps by administering it along with a physiologically acceptable reducing agent such as ascorbic acid.

Although these computer models are a gross simplification of the situation *in vivo*, for example we assume that all of the metals are in

TABLE 31

The selectivity of existing and proposed drugs for lead(II) over zinc(II)

(a)	edta	D-pen	cys	gsh
% zinc(II) complexed	33	10	9	14
% lead(II) complexed	50	100	100	94

(b)

glutathionate : lead(II)	2:1				1:1	3:1
[lead(II)] (μM)	3.0	7.0	10.0	15.0	10.0	10.0
% zinc(II) complexed	5	10	14	19	3	25
% lead(II) complexed	83	92	94	96	76	97

low molecular weight forms, they can narrow the field of possible therapeuticals from random screening down to 'key' molecules for animal studies. However, one can not always extrapolate *in vitro* calculations to the clinical treatment situation and exhaustive animal and clinical trials will always be necessary.

Structures of Some Lead(II) Complexes in Aqueous Solution

The thermodynamic functions for lead(II)-glycinate, -glycylglycinate, -glycylglycylglycinate, -cysteinate and -glutathionate complex formation, reported in Chapter 6, are used to suggest structures for the various complexes present in solution.

GLYCINATE AND ITS PEPTIDES

Evans and Monk [160], from potentiometric studies, suggested that the binding of lead(II) to glycine peptides was through the amino group and either the nitrogen or oxygen atom of the first peptide link. Rabenstein and Libich [122], from n.m.r. studies, and Nag and Banerjee [141], from polarography, show that at low pH the metal is bound to the carboxylate end of the ligand with the amino end still protonated and at high pH the amino end and the peptide oxygen are bound to the metal. Rabenstein and Libich consider that at high pH the carboxylate group is probably also bound to the metal and that polynuclear complex formation may be occurring. Their work also suggests that very little ionisation of the peptide hydrogen can be present.

From this work, table 18, we see that $\Delta H^{\ominus}_{110}$ values for glycinate and its peptides vary by less than 3 kJ mol^{-1}, the differences in formation constants being entropy determined. This indicates that the binding is the same in all three cases and so the peptides are probably bound by their amino and peptide oxygen groups. The large positive entropy change in the case of lead(II)-glycinate complexation reflects the more effective charge neutralisation caused by bonding of the carboxylate group, the smaller values for the peptides suggesting that their carboxylate groups are not bound to the metal.

There are two bonding possibilities for the 111 species found at lower pHs

(i) Binding of the glycinate by the carboxylate group to the lead(II) with the amino group protonated. For the protonation of the glycinate amino group we found $\Delta H^{\ominus}$ = -51.2 kJ mol^{-1} and for lead(II)-acetate complexation $\Delta H^{\ominus}$ = -0.25 kJ mol^{-1} has been reported [161]. So for this type of binding we expect $\Delta H^{\ominus}_{111} \sim$ -51.4 kJ mol^{-1}.

(ii) Binding of the glycinate by the amino group to the lead(II) with the carboxylate group protonated. For the protonation of the glycinate carboxylate group we found $\Delta H^{\ominus}$ = -9.0 kJ mol^{-1}. For the binding of cadmium(II) to ammonia or ethylamine $\Delta H \sim$ -14.8 kJ mol^{-1} [162,163] and we would expect the corresponding value for lead(II) to be similar. So for this type of bonding we would expect $\Delta H^{\ominus}_{111} \sim$ -23.8 kJ mol^{-1}.

The value found experimentally for $\Delta H^{\ominus}_{111}$ is -25.1 kJ mol^{-1} and so it would appear that the second type of bonding is occurring contrary to the view of Rabenstein and Libich [122].

In the species 111 in the case of glycylglycylglycinate it would appear that it is the free carboxylate group that is being protonated but for glycylglycinate $\Delta H^{\ominus}_{111}$ is more negative than we would expect.

The lead(II)-glycylglycinate system is also unusual in that a 11-1 species is not detected and a similar situation occurs in the case of zinc(II)-glycylglycinate [61]. The reason for these differences is not known.

In the lead(II)-glycinate 110 complex there are no ionisable protons other than those of coordinated water molecules which may ionise to give a lead(II)-hydroxy bond. For the formation of this bond Carell and Olin [37] found $\Delta H^{\ominus}$ between +20.9 and +41.8 kJ mol^{-1} and $\Delta S^{\ominus}$ between -81.1 and -1.1 J mol^{-1} K^{-1}. These can be compared with our values for the reaction 110→11-1 of $\Delta H^{\ominus}$ = +28.9 kJ mol^{-1} and $\Delta S^{\ominus}$ = -49.4 J mol^{-1} K^{-1} which certainly fall within these ranges.

In the lead(II)-glycylglycylglycinate system there is the possibility that ionisation of the peptide proton is occurring with binding of the peptide nitrogen to the metal. However, our values of $\Delta H^{\ominus}_{110\rightarrow 11-1}$ = +37.0 kJ mol^{-1} and of $\Delta S^{\ominus}_{110\rightarrow 11-1}$ = -16.8 J mol^{-1} K^{-1} still fall within the ranges for lead(II)-hydroxy bond formation.

CYSTEINATE

For the lead(II)-cysteinate 110 complex we find a high negative enthalpy (-42.2 kJ mol^{-1}) showing that bonding is not merely through the oxygen and nitrogen atoms as in the glycinate case but that the sulphur atom is also involved. The large positive value of $\Delta S^{\ominus}$ indicates that effective charge neutralisation has occurred and so both the sulphydryl and the carboxylate groups are probably bound to the metal. The tridentate nature of cysteinate towards lead(II) is also shown by its reluctance to form a 210 species whereas zinc(II) forms a 210 in preference to a 110 species[30]. Tridentate binding of cysteinate to lead(II) but not to zinc(II) has also been proposed by other workers [127-129,134,135].

For the reaction 110→111 we find $\Delta H^{\ominus}$ = -14.5 kJ mol^{-1} and there are three bonding possibilities

(i) Nitrogen and oxygen atoms bound to lead(II) with the sulphur atom protonated. For the binding of the glycinate N and O to lead(II) we have $\Delta H^{\ominus}$ = -12.4 kJ mol^{-1} and for the protonation of the cysteinate sulphydryl group we have $\Delta H^{\ominus}$ = -40.4 kJ mol^{-1}. So for this type of bonding we expect $\Delta H^{\ominus}_{111} \sim$ -52.8 kJ mol^{-1} and so $\Delta H^{\ominus}_{110\rightarrow111}$ to be approximately -10.4 kJ mol^{-1}.

(ii) Nitrogen and sulphur atoms bound to lead(II) with the carboxylate group protonated. For the reaction 110→111 for the glycinate case, where the carboxylate group is being protonated, we have $\Delta H^{\ominus}$ = -12.7 kJ mol^{-1} and we would expect a similar value here.

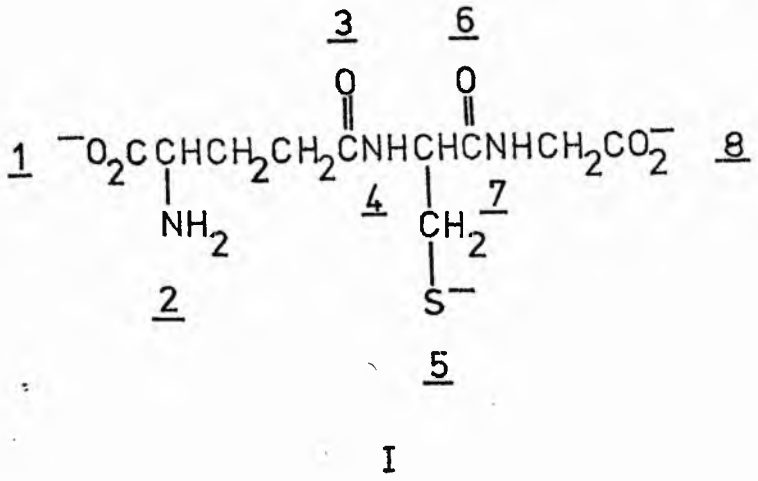
(iii) Sulphur and oxygen atoms bound to the lead(II) with the amino group protonated. We would expect $\Delta H^{\ominus}$ for the reaction 110→111 to be approximately equal to the enthalpy of protonation of the amino group (-38.8 kJ mol^{-1}) minus the value for the binding of the amino group to lead(II) (-14.8 kJ mol^{-1}) which gives approximately -24.0 kJ mol^{-1}.

Thus our experimental value is closest to that for carboxylate protonation but no definite assignment can be made.

For the 211 species, if we assume that one ligand is tridentate then this leaves $\Delta H^{\ominus}$ = -69.4 kJ mol^{-1} to be accounted for. This is closest to the expected value for the second ligand being bound to the metal through the sulphur and oxygen atoms with the amino group protonated i.e. -66.4 kJ mol^{-1} for (iii) rather than -52.8 kJ mol^{-1} for (i) or -55.1 kJ mol^{-1} for (ii).

GLUTATHIONATE

In the ligand glutathionate, I, there are eight sites through which bonding to the metal ion may occur.



I

Fuhr and Rabenstein [164] found no detectable binding of lead(II) to group 2, but only to 5 and to 1 and 8 in some regions of pH. For example, they found 8 bound up to high pH where it was replaced by a hydroxy group. Also they found no evidence for lead(II) promoted ionisation of the peptide protons.

The results reported here show $\Delta H^{\ominus}_{110}$ more negative than for lead(II)-cysteinate complex formation showing that more bonding is occurring. The negative value of $\Delta S^{\ominus}_{110}$ may be due to strain in the rings formed due to this multiple bonding. To obtain such a highly negative enthalpy of formation we believe, contrary to Fuhr and Rabenstein, that the amino group 2 must be bound to the metal ion along with 1 and 5. To make up the remaining enthalpy, group 8 may be involved, as they suggest, or alternatively the peptide links. We have shown from our studies of

glycine peptides that the oxygen of the peptide link rather than the nitrogen tends to bind to lead(II) and so groups 3 or 6 are more likely to be bound than 4 or 7.

For the reaction 110→111, for cysteinate we have $\Delta H^{\ominus}$ = -14.5 kJ mol^{-1} and for glutathionate $\Delta H^{\ominus}$ = -14.6 kJ mol^{-1} suggesting that the same process is occurring in each case, probably carboxylate protonation.

For the complex 211, $\Delta H^{\ominus}$ = -101.4 kJ mol^{-1} whereas $\Delta H^{\ominus}_{110} + \Delta H^{\ominus}_{111}$ = -149.8 kJ mol^{-1}. Thus, the bonding to the metal must be different for the second ligand with fewer groups being bound, presumably for steric reasons. If we assume that the first ligand is complexed as in the 111 species then the binding of the second ligand has to account only for $\Delta H^{\ominus}$ = -19.2 kJ mol^{-1} which is too low for the involvement of both the nitrogen and the sulphur atoms.

For the reaction 211→212, $\Delta H^{\ominus}$ = -42.1 kJ mol^{-1} which would correspond approximately to the protonation of a free nitrogen or sulphur atom on the second ligand.

Antirheumatoid Arthritis Drugs

It is suggested that antirheumatoid arthritis drugs may function by releasing copper(II) from serum albumin either by a direct competitive complexing mechanism or through a remote mechanism whereby the drug becomes bonded to a site some distance from the copper(II) ion. It may then facilitate the copper(II) release through allosteric effects,

possibly by distorting its binding site to a non-regular geometry so that circulating amino acid anions etc. can successfully compete with the protein.

The order of potency of the drugs in rat adjuvant arthritis is given [54] as

Indomethacin > Naproxen ≥ Ketoprofen > Fenoprofen ≥ Phenylbutazone > Aspirin (1)

and the order of potency against carrageenan induced rat foot oedema as [165]

Indomethacin > Naproxen ≥ Ketoprofen > Phenylbutazone > Aspirin > Fenoprofen (2)

These results are in remarkable agreement considering that (2) represents activity against an acute inflammatory response whereas (1) is thought to be a better mimic of chronic lesions.

The following conclusions can be drawn from the experimental results reported in Chapter 7.

(i) The order of copper(II)-ligand complexing at pH 3.9 is

histidine >> salicylic acid > Indomethacin ~ Fenoprofen > Naproxen ~ Ketoprofen > Aspirin > Phenylbutazone (3)

(ii) The order of copper(II)-ligand complexing at pH 7.4 is

histidine >> salicylic acid ~ Naproxen > Indomethacin > Fenoprofen ~ Ketoprofen ~ Phenylbutazone (4)

(iii) The ability of the drugs to release copper(II) from bovine serum albumin by remote action is

Fenoprofen ~ Phenylbutazone > Ketoprofen > Indomethacin ~ Aspirin ~ Naproxen (5)

(iv) The ability of the ligands to release copper(II) from bovine serum albumin by either mechanism is

histidine >> Indomethacin > Phenylbutazone > Ketoprofen (6)

~ Fenoprofen > Naproxen ~ salicylic acid > Aspirin

Clearly scheme (6) parallels scheme (1) with the exceptions of Naproxen and Phenylbutazone whose positions are reversed. This adds support to the suggestion that antirheumatoid arthritis drugs exhibit a serum albumin copper(II) releasing effect *in vivo*.

Both of the *in vitro* determinations of the copper(II) releasing properties of Naproxen and Phenylbutazone, (5) and (6), are in agreement concerning the relative efficacy of these agents but give the opposite order to the biological trials, (1) and (2). Perhaps these two drugs act on arthritis by a different mechanism to the others. The relative ineffectiveness of Phenylbutazone according to schemes (1) and (2) is not reflected by the large number of formulations of this agent listed in the British Pharmacopoeia. This suggests that perhaps the animal screening tests are not accurate models of rheumatoid arthritis.

Aspirin, as a copper(II) releaser from BSA directly, (3), or remotely, (5) and (6), is virtually useless and it also is placed very low in both potency orders, (1) and (2). However, Aspirin is by far the drug in most common use in rheumatoid arthritis treatment and so we see that much more information is required regarding its mode of action. There is the distinct possibility that Aspirin is converted into salicylic acid in plasma and then it could either have a direct copper(II) releasing effect, (3) and (4), or be removed as an analgesic functioning through the central nervous system. There have been literature reports [166,167] that it is the 25% of the salicylic

acid which is not bound to serum albumin that determines the drug efficacy.

Indomethacin, Fenoprofen and Ketoprofen are all capable of releasing bovine serum albumin bound copper(II) both remotely, (5) and (6), and directly, (3) and (4), although the latter mechanism is probably hindered by the competition from the high concentrations of amino acids present *in vivo*.

It would be of considerable interest to repeat these *in vitro* studies with human serum albumin and to obtain further objective evaluations of the relative drug potencies.

CHAPTER 9

"CHALLENGE" PROJECT - RESULTS AND DISCUSSION

CONTENTS

CHAPTER 9

"CHALLENGE" PROJECT - RESULTS AND DISCUSSION

Results

Formation constants for protonating glycinate and for nickel(II)-glycinate complexing were determined at 25.0°C, I = 1.00M$(Na^+)Cl^-$ as a contribution to the "challenge" project and the measurements were repeated with I = 1.00M$(Na^+)ClO_4^-$ in order to determine whether the background anion has a significant effect on the formation constants.

The concentration limits set for the "challenge" project were

nickel(II) concentration between 1.0 and 10.0mM and

glycinate to nickel(II) ratio less than or equal to 5 : 1.

The electrochemical cell used was

glass electrode	solution under study I = 1.00M$(Na^+)Cl^-$	1.00M Na Cl	AgCl(s) - Ag(s) 1.00M NaCl

This system was calibrated, for each background salt, as in Chapter 5, and pw_k was determined, as in Chapter 7, to give

$$pw_k \text{ (1.00M NaCl)} = 13.68 \quad \text{and}$$

$$pw_k \text{ (1.00M } NaClO_4\text{)} = 13.70$$

Our normal computational approach was adopted.

GLYCINATE PROTONATION

I = 1.00M(Na^+)Cl^-

Experimental results are shown in appendix 5 - table 1 and in figure 55.

The formation curves at different ligand concentrations are superimposable and the formation constants determined from these are shown in table 32(a).

I = 1.00M(Na^+)ClO_4^-

Experimental results are shown in appendix 5 - table 2 and in figure 56.

The calculated formation constants are shown in table 32(b).

TABLE 32

Log formation constants for glycinate - proton interactions at 25°C

	pqr	log β_{pqr}	n
(a) I = 1.00M(Na^+)Cl^-	101	9.6379 ± 0.0012	278
	102	12.0448 ± 0.0023	
(b) I = 1.00M(Na^+)ClO_4^-	101	9.6558 ± 0.0027	329
	102	12.1232 ± 0.0054	

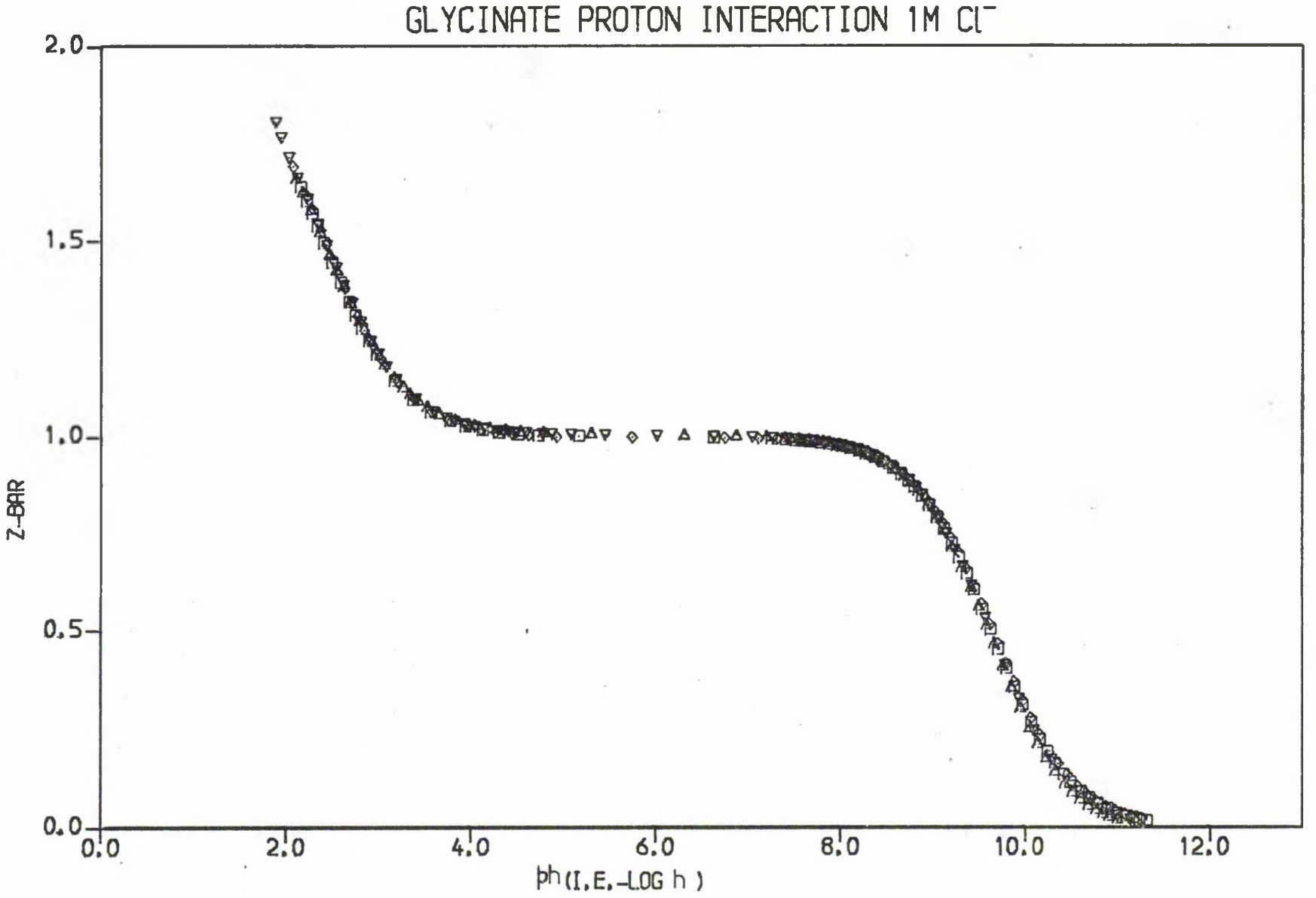


FIGURE 55 : ZPLOT OF DATA FROM APPENDIX 5 - TABLE 1

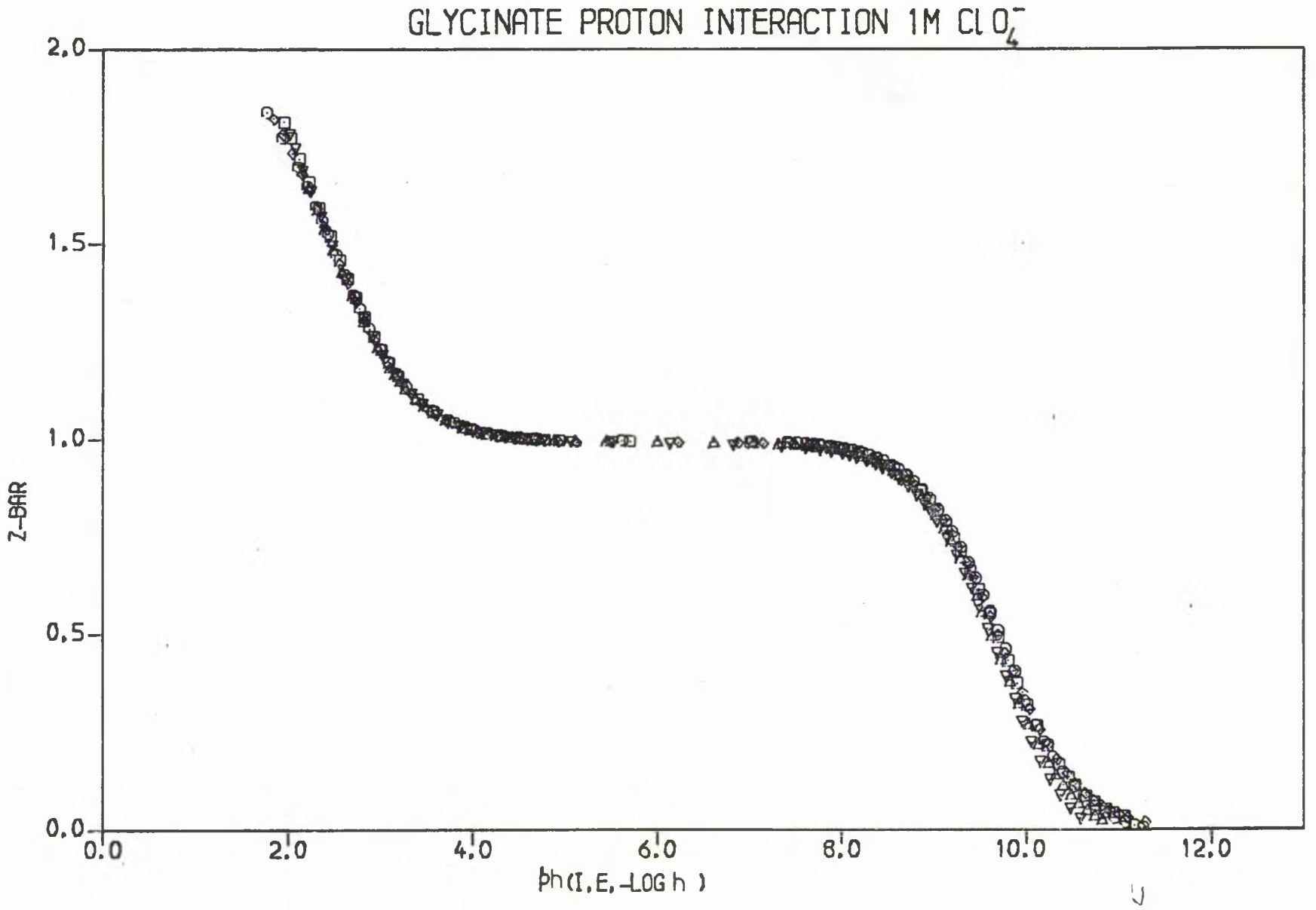


FIGURE 56 : ZPLOT OF DATA FROM APPENDIX 5 - TABLE 2

NICKEL(II)-GLYCINATE INTERACTION

I = 1.00M(Na^+)Cl^-

Experimental results are shown in appendix 5 - table 3 and in figure 57.

A spread of formation curves is found depending on the glycinate to nickel(II) ratio. The 'curl-backs' found at high $\bar{Z}$ indicate the presence of hydroxy species.

The complexes searched for were those with p 0 to 3; q 1 and r -2 to 1, those giving convergence being 110, 210, 310, 11-1, 21-1, 31-1 and 01-1 with log β values of 5.813, 10.581, 14.318, -3.30, -0.65, 3.72 and -8.32 respectively. Arnek's [168] 04-4 complex was offered for computer refinement but no convergence was achieved.

A COMPLOT model of the system, figure 58, shows that the complexes 21-1, 31-1 and 01-1 are present only in small amounts at high pH and so the values obtained for their formation constants will not be reliable.

The computed log formation constants, their standard deviations and the number of experimental observations are given in table 33(a). A simulated set of formation curves obtained from PSEUDOPLOT using these formation constants is shown in figure 59 and gives a very good fit to the experimental curves.

I = 1.00M(Na^+)ClO_4^-

Experimental results are shown in appendix 5 - table 4 and in figure 60.

These formation curves are very similar to those found in the other ionic background and the same complexes were searched for. Those which gave convergence were 110, 210, 310, 11-1, 21-1, 31-1 and

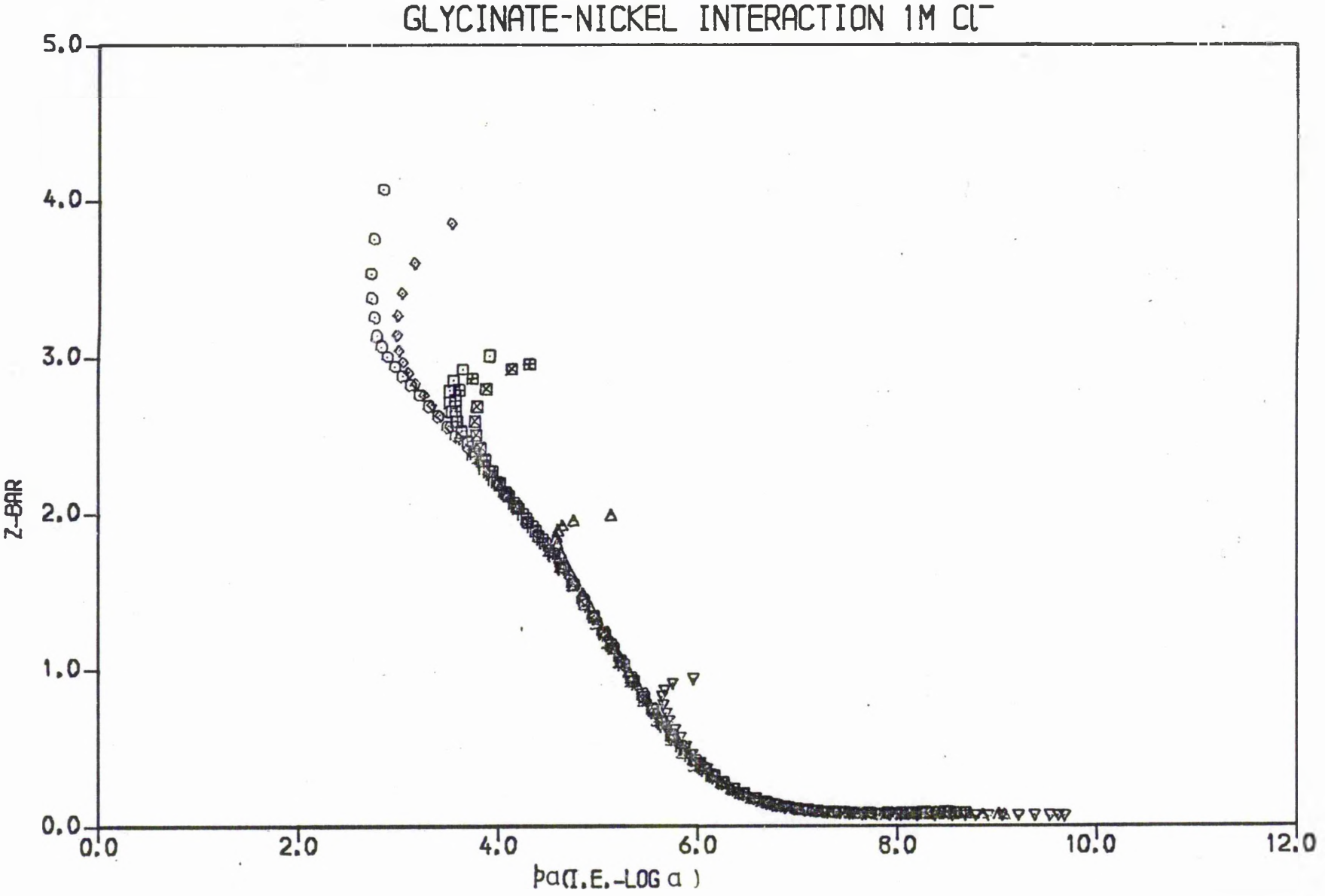


FIGURE 57 : ZPLOT OF DATA FROM APPENDIX 5 - TABLE 3

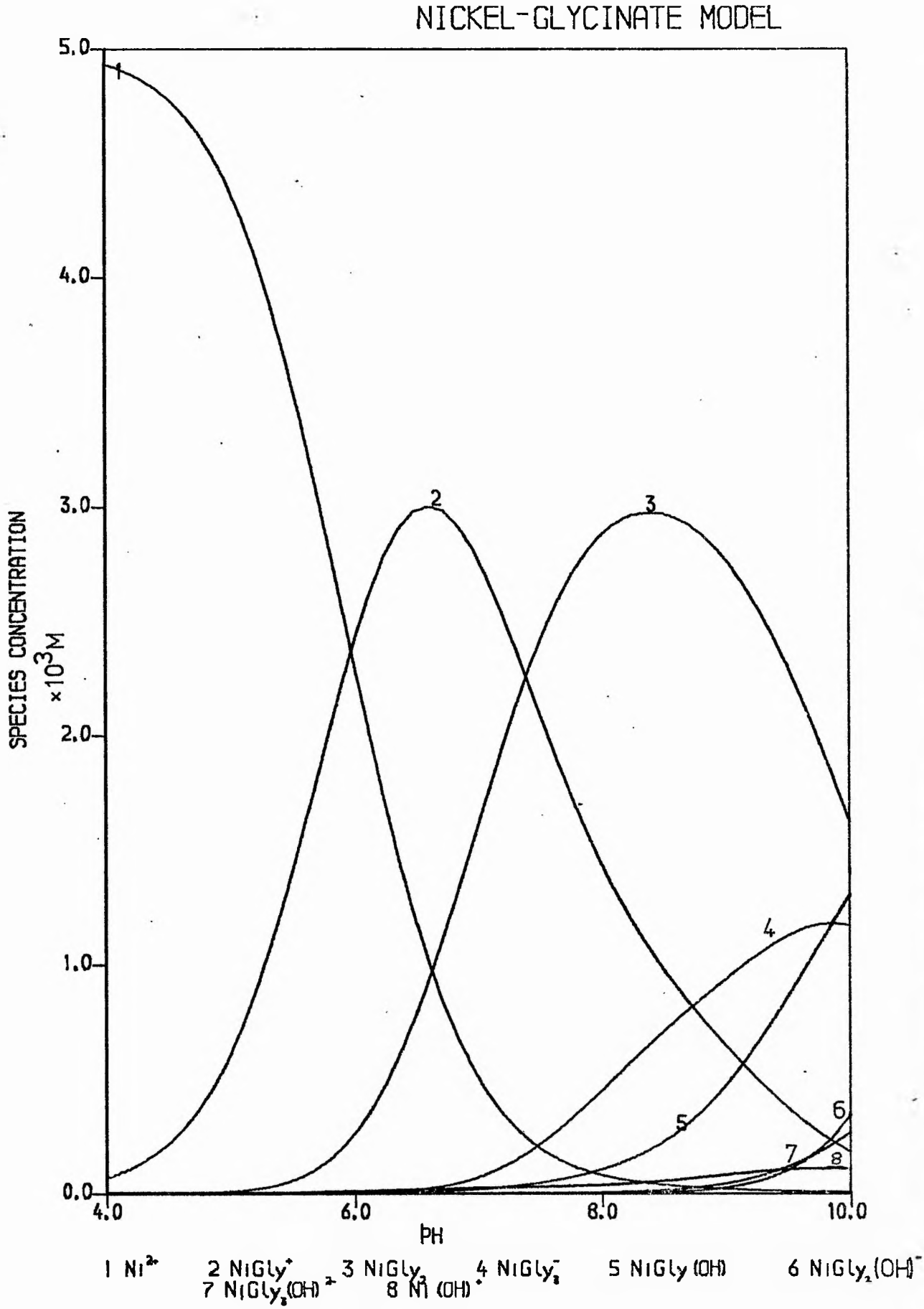


FIGURE 58 : A COMPLOT MODEL USING THE FORMATION CONSTANTS FROM TABLE 33(a) PLUS LOG $\beta_{21-1} = -0.65$, LOG $\beta_{31-1} = 3.72$ AND LOG $\beta_{01-1} = -8.32$

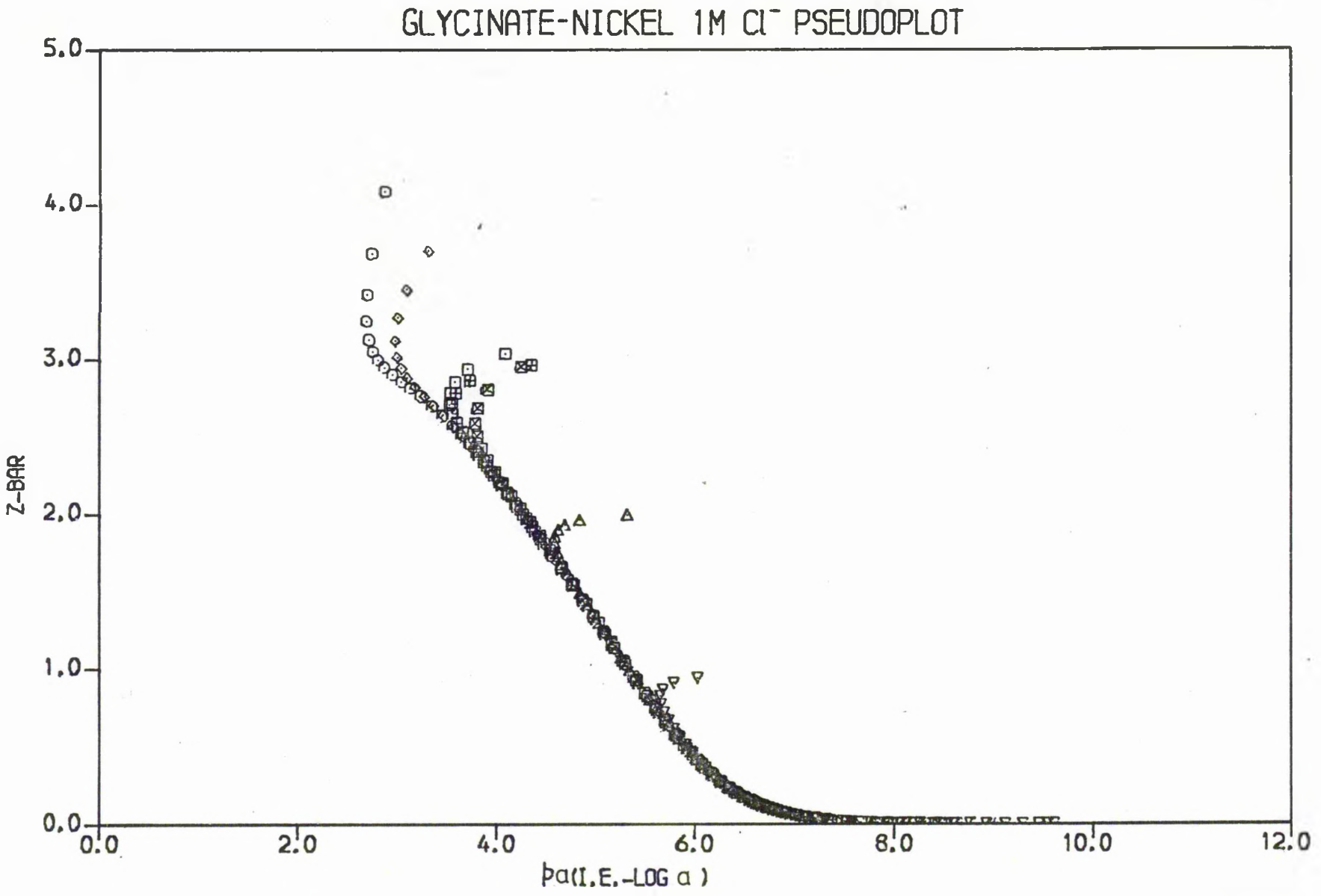


FIGURE 59 : PSEUDOPLOT USING FORMATION CONSTANTS FROM TABLE 33(a)

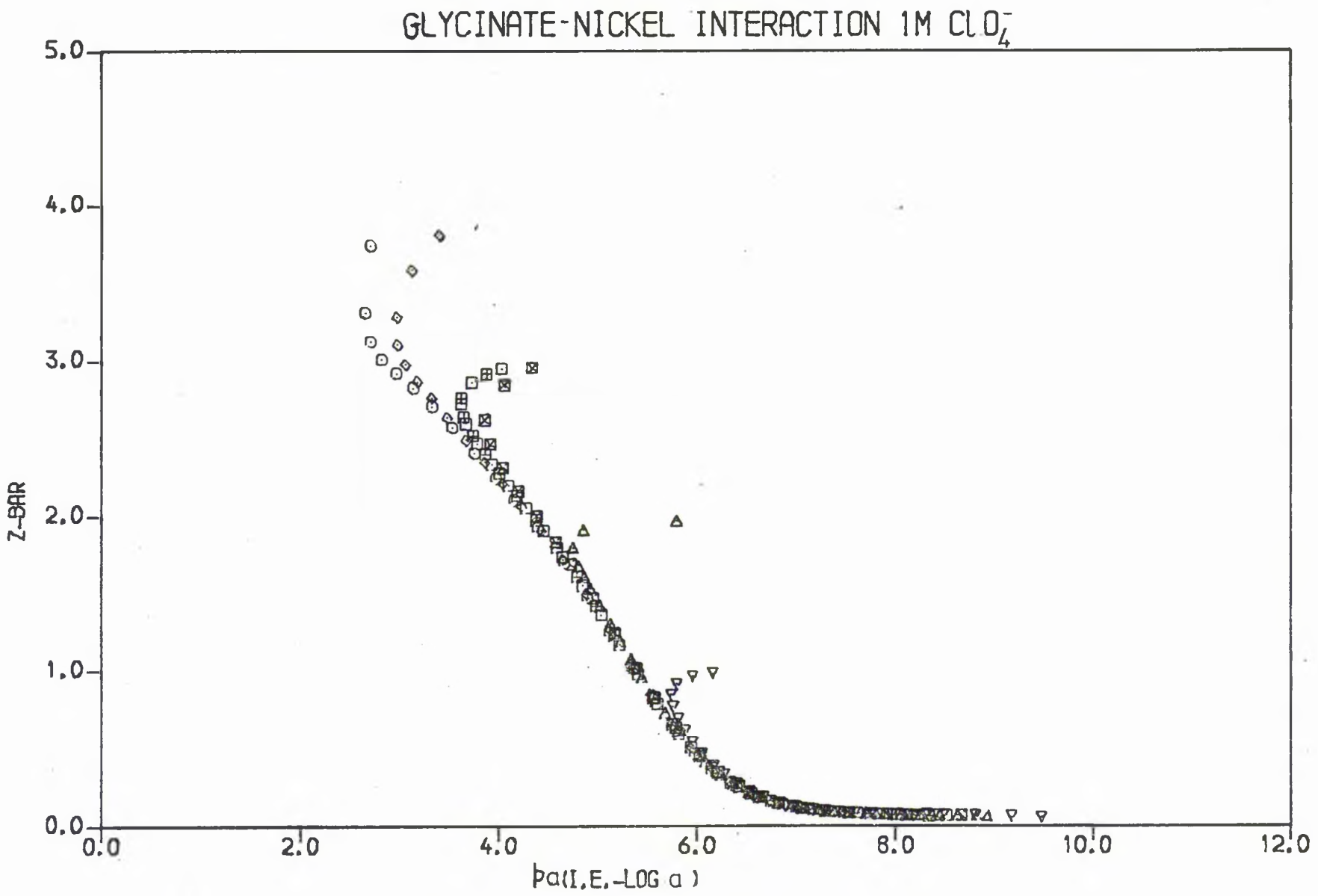


FIGURE 60 : ZPLOT OF DATA FROM APPENDIX 5 - TABLE 4

01-1 with logβ values of 5.895, 10.728, 14.516, -3.00, 0.61, 3.56 and -8.07 respectively. Again a COMPLOT model shows that the last three of these complexes are not significant.

The results are summarised in table 33(b) and a simulated set of formation curves obtained from PSEUDOPLOT using these constants is shown in figure 61.

TABLE 33

Log formation constants for nickel(II)-glycinate complexes at 25°C

	pqr	log β_{pqr}	n
(a) I = 1.00M(Na^+)Cl^-	110	5.813 ± 0.020	349
	210	10.581 ± 0.030	
	310	14.318 ± 0.052	
	11-1	-3.30 ± 0.43	
(b) I = 1.00M(Na^+)ClO_4^-	110	5.895 ± 0.016	199
	210	10.728 ± 0.026	
	310	14.516 ± 0.047	
	11-1	-3.00 ± 0.35	

Thus it would seem that formation constants are lowered slightly by the use of chloride as the background anion.

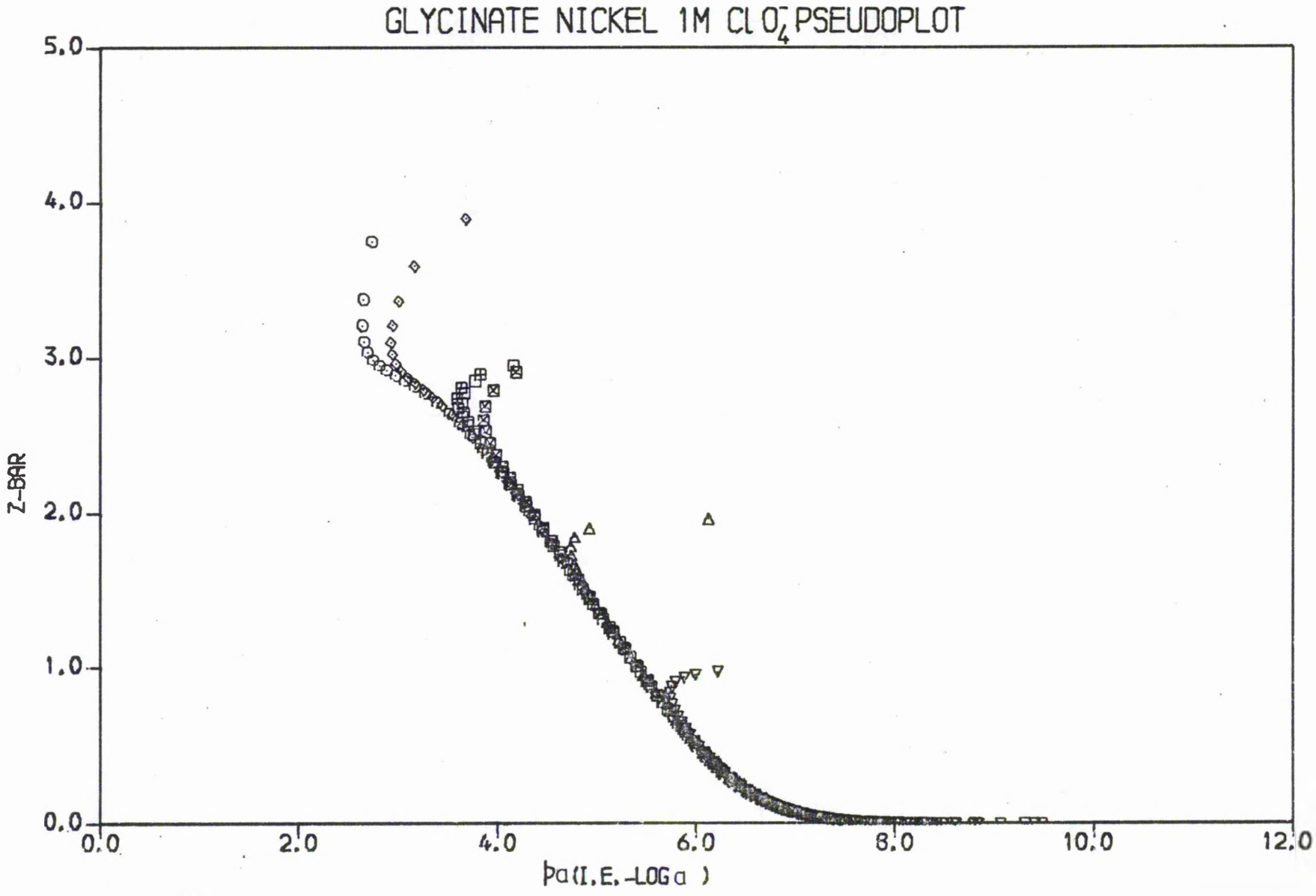


FIGURE 61 : PSEUDOPLOT USING FORMATION CONSTANTS FROM TABLE 33(b)

Discussion

Groups of workers from the Italian Universities of Florence, Naples, Parma, Rome and Turin are taking part in the "challenge" project. Results are not yet available from all of the groups but those that are are summarised in tables 34 and 35.

Detailed experimental data is available only from Professor Paoletti's group in Florence and this has been used in our ZPLOT program to give the formation curves shown in figure 62. These results show

(i) curves of a lower gradient than this work

(ii) none of the 'curl-backs' found in this work

(iii) one displaced curve

(iv) the curve levelling off at $\bar{Z} = 3.0$ has been achieved by using concentrations outside the originally set limits i.e. glycinate to nickel(II) ratio of 6 : 1.

The displaced curve, which was at the very high glycinate to nickel(II) ratio of 11 : 1, can probably be assigned to an analytical or card punching error. However, a much more serious discrepancy is the lack of 'curl-back' on the Florence curves which in our work leads to a formation constant for the 11-1 complex.

In St Andrews several repeat titrations and variations in the computational input have been carried out but no experimental error which could have been responsible for causing the 'curl-backs' has been found.

For the lower ratios of glycinate to nickel(II) the Florence titrations have been stopped at pHs less than 7 and no points at

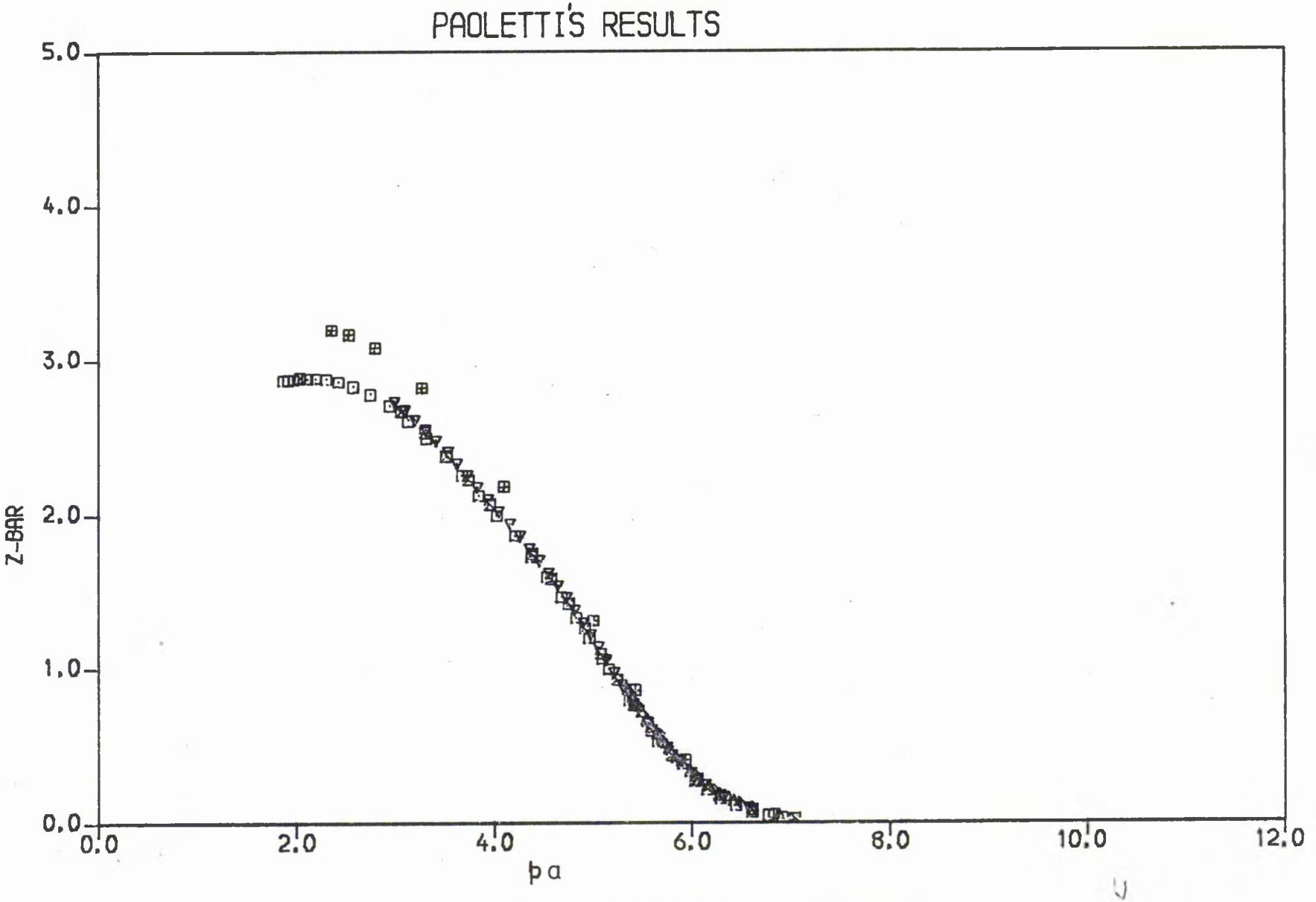


FIGURE 62 : ZPLOT OF DATA FROM FLORENCE

TABLE 34

"Challenge" project - reagents and apparatus

	ST ANDREWS	CATANIA	FLORENCE	TURIN
Reagents and analyses	NaCl (Fisons A.R.) dried at 200°C $NiCl_2 6H_2O$ analysed by edta and electro-deposition Gly (Fisons A.R.)	NaCl (C. Erba R.P.) dried $NiCl_2 6H_2O$ (C. Erba R.P.) analysed Gly (C. Erba R.P.)	NaCl (Merck superpure) $NiCl_2 6H_2O$ analysed gravimetrically by dimethylglyoxime Gly (Merck)	NaCl prepared by bubbling HCl into NaOH $NiCl_2$ prepared from the basic carbonate + HCl and analysed photometrically
Meter	Solartron LM1867	Radiometer PHM52	Radiometer PHM4A	Metrohm E338
Reference electrode	Ag/AgCl	Saturated calomel	Ag/AgCl	Saturated calomel
Measuring electrode	Glass (Russell pH)	Glass (Ingold)	Glass (Orion 91-01-00)	Glass (Metrohm EA109/7)
Liquid junction	Salt bridge, 1M NaCl	-	Wilhelm bridge, 1M NaCl	Wilhelm bridge, 1M NaCl
Burette	Piston (Metrohm E274)	Piston (Metrohm Dosimat E412)	Piston (Metrohm Dosimat E415)	Microsyringe
Other conditions	Bubbling N_2 through solution. Both cells at 25°C.	Bubbling N_2 through solution. Single cell at 25°C.	N_2 on surface. Both cells and room at 25°C.	N_2. Both cells and bridge at 25°C.

TABLE 35

"Challenge" project -

$\log \beta_{pqr}$

pqr	ST ANDREWS	CATANIA
101	9.6379 ± 0.0012	9.652 ± 0.012
102	12.0448 ± 0.0023	12.110 ± 0.010
110	5.813 ± 0.020	5.600 ± 0.015
210	10.581 ± 0.030	10.325 ± 0.025
310	14.318 ± 0.052	13.650 ± 0.040
11-1	-3.30 ± 0.43	

- results

FLORENCE	ROME	TURIN
9.6535 ± 0.0024	9.67 ± 0.04	9.656 ± 0.014
12.0666 ± 0.0033	12.14 ± 0.06	12.076 ± 0.010
5.6247 ± 0.0045	5.53 ± 0.03	5.625 ± 0.01
10.3555 ± 0.0070	10.26 ± 0.04	10.398 ± 0.04
13.7538 ± 0.1251	13.58 ± 0.05	13.911 ± 0.087

pH >9.5 have been considered. Thus, probably, the titrations have been stopped before the 'curl-backs' occurred. The question now arises - why was this the case?

In the work reported in this thesis a white lamp and light scattering was used to detect precipitates and a titration was discontinued as soon as precipitation occurred. No drifting in the emf readings was seen before the first sighting of precipitate.

Thus we believe that the data points in the 'curl-back' regions do represent a true solution at equilibrium and so should be included in the computational analysis to give a formation constant for the 11-1 complex.

CHAPTER 10

CONCLUSION

CHAPTER 10

CONCLUSION

As we have seen, the average concentration of lead in blood plasma and its clinical threshold are very close to each other and so, in comparison to the low concentration of the *in vivo* essential metals, relatively large quantities of lead may have to be removed. The accepted treatment for plumbism employs the calcium disodium salt of ethylenediaminetetraacetate and D-penicillamine but these are not particularly selective.

We have thus suggested glutathione as an alternative therapeutical and a considerable amount of work can still be done on this ligand *in vitro*. Lead removal requires the presence of neutral complexes for effective lipid-protein membrane solubility and permeability and COMPLOT models have shown that these are present in the lead(II)-glutathionate system. However, partition studies involving *n*-octanol, or another membrane model, would give a further indication of the *in vivo* behaviour of lead- and essential metal-glutathionate complexes. The use of a microcalorimeter would allow more accurate enthalpy determinations and so improve structural predictions.

Animal experiments would then be necessary to establish such factors as toxicity, dose-response relationships and excretion rates.

Further *in vitro* work to find other lead specific ligands which would be non-toxic *in vivo* would be of value, for example, the determination of the formation constants of the complexes of other short peptides containing a cysteinate residue.

Much more work on the interaction of drugs and metals with serum proteins and on the composition of biological fluids and tissues, in normal and pathological states, is also necessary in order to allow us to improve the realism of our biological models. This inclusion of protein binding necessitates a considerable amount of alteration to computer programs to allow them to deal with such a large number of electron donor groups and requires accurate amino acid analyses of the major serum proteins.

As far as the studies on rheumatoid arthritis are concerned, more realistic animal models are required to compare the efficacies of the drugs and double-blind trials on rheumatoid arthritis patients themselves are necessary. In general, there should be an awareness that many drugs interact with serum albumin and so may effect the binding of metal ions to this protein. Even the mode of action of Aspirin, a very widely used drug, is not adequately understood.

We must remember that biological models can only be as accurate as the data used to compute them and there is a great need for accurate formation constants determined under standard conditions. However, at the same time, approximate formation constants are better than none at all.

Further interlaboratory cooperation and the publication of detailed experimental data will enable steps to be made towards improving techniques and so ultimately achieving more accurate models of

biological systems. These models, of course, are very far from completely predicting *in vivo* behaviour but they can, at least, suggest hopeful areas for clinical studies.

APPENDIX 1 : EXPERIMENTAL DATA FOR LIGAND PROTONATION CONSTANT DETERMINATION

Table 1 EXPERIMENTAL DATA FOR GLUTAMATE PROTONATION

Titn. No.	Starting Solution A mM	Starting Solution B mM	Starting Solution H mM	Titrating Solution A mM	Titrating Solution B mM	Titrating Solution H mM	Initial Vol. ml	E° mV
1	19.96		21.49	19.98		-80.06	20.01	451.1
2	9.979		10.75	9.988		-40.02	20.01	452.5
3	10.06		21.48	9.973		-80.10	20.01	452.5
4	5.031		10.74	4.990		-40.08	20.01	451.1
5	10.06		-40.05	10.10		21.48	20.01	451.1

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.20	322.6	8.40	190.2	13.75	48.8	17.00	-109.6
1.00	312.2	9.30	179.4	13.80	26.6	18.50	-122.5
1.80	301.2	10.00	170.9	13.82	16.5	20.00	-132.7
2.60	289.6	10.90	159.5	13.85	2.4	22.00	-144.8
3.50	275.5	11.60	149.3	13.88	-8.0	24.00	-156.5
4.10	265.0	12.20	138.7	13.95	-24.3	26.00	-168.7
4.70	253.2	12.70	127.2	14.05	-38.0	28.00	-183.1
5.20	243.1	13.00	118.0	14.20	-50.8	30.00	-200.5
5.80	230.7	13.30	104.7	14.40	-61.8	32.10	-219.6
6.30	221.0	13.50	90.7	14.70	-72.8		
6.90	210.9	13.60	80.1	15.20	-85.1		
7.60	200.6	13.70	62.7	16.00	-98.1		
Titration No. 2							
0.10	312.5	9.40	177.4	13.75	28.6	16.30	-100.6
1.20	300.8	10.00	170.3	13.78	14.0	17.30	-110.9
2.40	286.4	11.00	157.5	13.81	1.5	18.70	-122.1
3.50	271.0	12.00	141.8	13.85	-11.0	20.00	-130.8
4.30	257.6	12.70	125.8	13.90	-21.9	22.00	-142.6
5.10	242.1	13.20	107.1	14.00	-35.8	24.60	-157.1
5.80	228.3	13.45	90.2	14.20	-52.0	27.00	-171.0

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
6.50	215.7	13.60	72.0	14.40	-61.9	30.00	-190.8
7.20	205.0	13.65	62.4	14.80	-75.2	32.50	-207.2
8.20	191.8	13.70	48.6	15.40	-87.9		
Titration No. 3							
0.10	342.4	5.90	219.1	9.00	69.7	10.40	-113.5
1.00	333.2	6.30	206.0	9.05	24.7	11.00	-127.3
2.00	320.4	6.80	191.8	9.10	-21.0	11.80	-143.5
2.80	307.7	7.30	178.3	9.15	-39.0	12.60	-160.1
3.50	294.3	7.90	160.9	9.20	-49.7	13.20	-174.5
4.10	280.8	8.40	141.4	9.30	-63.5	13.90	-194.6
4.60	266.9	8.70	122.8	9.40	-72.8		
5.10	249.8	8.90	98.4	9.60	-85.5		
5.50	234.0	8.95	87.7	10.00	-101.8		
Titration No. 4							
0.10	324.9	6.20	205.4	8.95	68.5	10.00	-103.1
0.50	321.5	6.60	193.9	9.00	35.1	10.40	-114.0
2.00	305.9	7.00	183.3	9.05	-14.1	11.00	-127.4
3.10	290.5	7.50	169.6	9.10	-35.6	12.10	-148.5
3.90	276.1	7.90	157.4	9.15	-47.5	13.00	-165.9
4.60	258.9	8.30	141.9	9.20	-55.7	13.80	-183.2
5.00	246.6	8.60	124.4	9.30	-67.4		
5.40	232.2	8.80	104.6	9.50	-82.3		
5.80	218.1	8.90	85.6	9.70	-92.0		
Titration No. 5							
9.50	-189.6	17.00	-88.0	18.90	62.4	23.00	160.8
10.00	-179.0	17.50	-76.9	19.10	83.3	24.50	171.6
10.50	-169.7	18.00	-60.8	19.30	96.6	26.50	183.5

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont,							
11.00	-161.8	18.30	-43.8	19.50	106.2	29.00	196.5
12.00	-148.8	18.50	-21.7	19.80	116.5	31.00	206.6
13.00	-137.6	18.60	0.4	20.00	121.8	33.30	218.1
14.00	-126.9	18.70	30.5	20.50	132.3		
15.00	-116.0	18.75	42.7	21.20	142.8		
16.00	-103.8	18.80	51.9	22.00	151.9		

Table 2 EXPERIMENTAL DATA FOR ETHYLENEDIAMINETETRAACETATE STANDARDIZATION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	10.00		24.94	10.00		-50.04	20.01	430.8
2	5.001		12.47	5.005		-25.04	20.01	432.3
3	30.00		49.91	29.98		-200.2	20.01	432.7
4	7.987		-40.06			124.6	20.01	434.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	309.8	9.05	220.7	10.17	85.3	15.50	-13.4
1.00	304.5	9.20	214.0	10.25	80.7	15.90	-19.5
1.60	300.8	9.30	208.3	10.45	71.5	16.30	-26.2
2.40	295.9	9.35	204.8	10.60	66.1	16.60	-31.3
3.20	290.9	9.40	201.1	10.80	59.9	16.90	-36.6
4.00	285.6	9.45	196.6	11.00	54.7	17.20	-41.8
4.80	279.9	9.50	191.4	11.20	50.1	17.50	-47.2

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
5.40	275.3	9.55	185.1	11.40	45.9	17.80	-52.1
5.90	271.1	9.59	178.9	11.65	41.1	18.10	-56.9
6.50	265.5	9.62	173.4	11.95	36.0	18.45	-62.2
7.00	260.3	9.66	164.4	12.30	30.5	18.80	-66.9
7.40	255.5	9.70	153.7	12.60	26.1	19.20	-72.0
7.80	249.9	9.74	143.1	13.00	20.7	19.60	-76.6
8.10	245.0	9.78	132.6	13.40	15.2	20.00	-80.9
8.35	240.3	9.82	123.4	13.90	8.7	20.50	-85.9
8.55	235.8	9.88	112.8	14.30	3.4	21.00	-90.5
8.75	230.7	9.95	103.7	14.70	-2.0		
8.95	224.5	10.05	93.9	15.10	-7.6		
Titration No. 2							
0.10	299.1	8.60	227.8	11.15	51.2	17.70	-49.3
0.50	297.2	8.75	223.7	11.40	46.0	18.05	-55.0
1.00	294.6	8.90	218.8	11.70	40.6	18.40	-60.2
1.60	291.5	9.00	215.1	12.00	35.6	18.80	-65.7
2.20	288.3	9.10	210.7	12.35	30.2	19.60	-75.3
2.70	285.6	9.20	205.5	12.75	24.5	20.00	-79.5
3.40	281.5	9.30	199.1	13.15	19.1	21.00	-89.0
4.10	277.4	9.45	185.7	13.55	13.8	22.00	-97.5
4.70	273.4	9.55	172.1	13.95	8.6	23.00	-105.4
5.10	270.6	9.65	151.7	14.35	3.4	24.00	-113.1
5.60	266.8	9.75	128.3	14.75	-1.9	25.00	-120.8
6.10	262.7	9.85	111.3	15.20	-8.2	26.00	-129.0
6.70	257.0	9.95	99.9	15.65	-14.9	27.00	-137.9
7.20	251.5	10.05	91.4	16.05	-21.1	28.00	-148.3
7.60	246.4	10.20	81.9	16.40	-26.9	29.00	-161.2
8.00	240.3	10.40	72.7	16.75	-32.9	30.00	-177.7

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
8.30	234.7	10.75	61.1	17.10	-39.0		
8.45	231.4	10.95	55.8	17.40	-44.3		
Titration No. 3							
0.10	303.7	4.80	173.2	10.50	-74.7	14.80	-156.9
0.40	300.5	4.85	150.3	10.70	-78.8	14.95	-162.9
0.70	297.3	4.88	134.3	11.00	-84.4	15.05	-167.5
1.00	294.0	4.91	122.5	11.20	-88.1	15.15	-173.0
1.30	290.6	4.98	104.8	11.45	-92.4	15.20	-176.0
1.60	287.2	5.04	95.0	11.70	-96.4	15.25	-179.4
1.90	283.5	5.18	80.2	11.95	-100.4	15.30	-183.0
2.20	279.7	5.45	63.0	12.20	-104.4	15.35	-187.1
2.50	275.5	5.80	48.9	12.50	-109.2	15.40	-191.7
2.80	271.0	6.50	29.1	12.75	-113.2	15.45	-196.3
3.00	267.8	7.20	13.0	13.00	-117.2	15.50	-201.7
3.20	264.2	8.20	-10.4	13.25	-121.5	15.55	-206.9
3.40	260.3	9.20	-39.8	13.50	-125.9	15.60	-212.2
3.60	255.9	9.80	-57.8	13.75	-130.6	15.70	-222.4
3.80	250.9	9.90	-60.5	14.00	-135.6	15.80	-230.4
4.35	230.4	10.00	-63.1	14.20	-140.2		
4.60	211.9	10.15	-66.8	14.40	-145.2		
4.75	188.5	10.35	-71.5	14.60	-150.9		
Titration No. 4							
3.85	-172.3	5.20	-32.5	6.51	145.1	7.40	268.3
3.88	-165.2	5.40	-9.6	6.52	156.6	7.70	276.4
3.92	-157.0	5.60	8.1	6.53	166.5	8.10	284.7
3.96	-150.0	5.72	17.5	6.54	175.6	8.60	292.7
4.01	-142.4	5.85	27.0	6.55	182.7	9.20	300.4

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 4 cont,							
4.07	-134.9	5.98	37.0	6.57	194.3	10.00	308.5
4.14	-127.3	6.13	49.9	6.59	202.4	10.80	315.1
4.24	-118.1	6.23	60.3	6.62	211.2	11.70	321.2
4.35	-109.1	6.33	74.0	6.67	221.3	12.70	326.7
4.47	-100.2	6.40	87.6	6.73	230.2	14.00	332.5
4.60	-90.6	6.45	102.9	6.81	238.7	15.50	337.7
4.73	-80.6	6.48	118.2	6.91	246.4	17.50	343.1
4.86	-69.9	6.49	125.9	7.05	254.6		
4.98	-58.5	6.50	134.7	7.20	261.3		

Table 3 EXPERIMENTAL DATA FOR GLUTATHIONATE PROTONATION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	9.986		12.47	9.986		-30.04	20.01	453.1
2	4.967		12.47	4.986		-60.08	20.01	453.1
3	8.039		24.94	8.010		-150.1	20.01	453.4
4	1.998		12.47	2.002		-60.08	20.01	453.5

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.20	318.6	11.00	241.5	21.00	154.6	22.45	19.9
1.00	313.1	12.00	235.2	21.30	145.5	22.60	10.5
1.50	309.6	13.00	229.0	21.60	132.5	22.80	1.6
2.00	305.9	14.00	222.8	21.80	119.2	23.10	-7.9
3.00	298.5	15.00	216.5	21.90	109.3	23.50	-17.0

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
4.00	291.1	16.00	209.9	22.00	94.9	24.00	-25.0
5.00	283.6	17.00	202.8	22.05	84.8	25.00	-36.2
6.00	276.1	18.00	194.9	22.10	72.9	26.00	-44.1
7.00	268.8	19.00	185.5	22.15	60.8	28.00	-55.1
8.00	261.6	19.80	176.1	22.20	49.9	30.00	-62.8
9.00	254.7	20.00	173.4	22.25	41.5		
10.00	247.9	20.50	165.3	22.35	28.9		
Titration No. 2							
0.20	330.3	5.20	218.8	6.30	-3.2	10.00	-129.2
1.00	321.0	5.40	210.3	6.35	-14.9	10.60	-142.3
2.00	306.1	5.60	200.2	6.40	-23.1	11.20	-158.3
2.50	296.7	5.80	187.5	6.50	-34.5	11.60	-172.1
3.00	286.1	5.90	179.0	6.60	-42.7	11.90	-183.9
3.50	273.4	6.00	167.5	6.80	-54.3	12.10	-191.8
3.90	262.3	6.10	148.0	7.00	-62.9	12.40	-202.5
4.20	253.2	6.15	130.0	7.40	-76.2	12.70	-210.8
4.50	243.5	6.20	79.9	7.80	-86.2	13.10	-219.1
4.50	243.5	6.22	44.7	8.30	-97.0		
4.80	233.6	6.25	17.2	8.90	-108.5		
5.00	226.6	6.27	7.4	9.50	-119.7		
Titration No. 3							
0.20	348.8	3.50	245.9	4.65	-20.2	7.00	-143.4
0.60	343.1	3.70	234.8	4.70	-31.2	7.10	-148.4
1.00	336.3	3.90	222.9	4.75	-39.1	7.20	-154.0
1.40	328.0	4.00	216.3	4.85	-50.2	7.30	-160.2
1.80	318.1	4.10	208.7	4.95	-58.6	7.40	-167.4
2.10	308.9	4.20	199.9	5.10	-68.6	7.50	-175.6

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
2.30	301.9	4.30	189.0	5.30	-79.1	7.60	-185.0
2.50	294.2	4.40	173.1	5.50	-87.9	7.70	-194.6
2.70	285.9	4.50	139.9	5.80	-99.0	7.80	-203.8
2.90	277.0	4.54	87.4	6.10	-109.5	7.90	-211.8
3.11	266.8	4.58	9.7	6.40	-120.0	8.10	-223.8
3.30	256.9	4.60	-2.2	6.70	-130.9	8.30	-232.3
Titration No. 4							
2.00	315.7	4.55	210.5	4.98	-30.4	6.10	-122.9
2.50	306.7	4.65	197.5	5.02	-40.2	6.30	-135.1
3.00	295.2	4.70	189.6	5.06	-47.7	6.45	-145.6
3.40	282.5	4.75	179.1	5.10	-53.8	6.55	-153.5
3.60	274.6	4.80	163.3	5.15	-60.0	6.65	-162.2
3.80	265.3	4.84	140.1	5.20	-65.5	6.75	-171.1
4.00	254.2	4.86	115.1	5.30	-74.6	6.85	-181.2
4.15	244.4	4.88	57.3	5.40	-82.1	6.95	-189.2
4.25	237.1	4.90	13.2	5.55	-92.0	7.05	-196.0
4.35	229.3	4.92	-4.9	5.70	-100.6	7.20	-204.1
4.45	220.6	4.95	-20.2	5.90	-111.6		

Table 4 EXPERIMENTAL DATA FOR GLYCINATE PROTONATION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	39.90		26.83			-39.90	24.98	457.7
2	39.90		26.83			-39.90	24.98	457.7
3	40.00		-39.90			53.60	24.98	410.3

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.0	307.8	7.00	282.0	18.40	-62.1	28.00	-133.0
1.00	304.5	8.00	277.9	19.00	-73.8	30.00	-140.9
2.00	300.8	10.00	268.9	20.00	-86.8	32.00	-148.5
3.00	297.1	14.00	243.4	21.00	-95.9	34.00	-156.3
4.00	293.4	16.00	215.5	22.00	-103.3	36.00	-164.6
5.00	289.7	18.00	-49.8	24.00	-115.1		
6.00	285.9	18.10	-53.6	26.00	-124.6		
Titration No. 2							
0.0	308.1	16.85	166.3	17.20	4.8	22.00	-105.4
2.00	300.7	16.90	158.4	17.22	-0.4	24.10	-117.4
4.00	293.4	16.95	148.0	17.25	-6.6	26.00	-126.3
8.00	277.8	17.00	133.0	17.30	-14.6	28.00	-134.6
12.00	257.8	17.05	108.8	17.40	-26.0	30.00	-142.4
14.00	242.5	17.07	93.3	17.60	-39.7	33.00	-153.9
15.00	231.0	17.08	83.4	17.80	-48.8	36.00	-166.2
15.60	221.2	17.10	63.9	18.00	-55.8	38.00	-175.4
16.00	212.3	17.12	45.6	18.40	-66.1	40.00	-186.1
16.35	200.9	17.14	30.1	19.00	-76.6		
16.55	191.6	17.16	19.6	20.00	-88.8		
16.70	181.7	17.18	10.9	21.00	-98.1		

Table 4 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3							
0.0	-260.1	14.50	-151.3	18.04	-5.0	18.60	137.0
0.50	-253.3	15.50	-140.4	18.06	2.5	18.70	145.5
1.50	-241.4	16.50	-124.6	18.08	9.3	18.90	157.3
2.50	-231.6	16.90	-115.4	18.10	14.6	19.20	169.0
3.00	-227.2	17.20	-107.2	18.15	29.2	19.50	177.2
4.00	-219.6	17.50	-95.3	18.20	42.9	20.00	187.1
5.50	-209.6	17.65	-86.7	18.25	57.2	20.50	194.4
6.50	-201.9	17.80	-72.4	18.30	72.7	21.00	200.4
8.00	-193.6	17.90	-56.3	18.35	89.3	22.00	210.1
10.00	-182.5	17.95	-43.2	18.40	104.5	24.10	224.8
12.00	-170.6	18.00	-23.4	18.45	116.1	26.10	235.5
13.00	-163.7	18.02	-13.9	18.50	124.8	28.00	244.1

Table 5 EXPERIMENTAL DATA FOR GLYCYLGLYCINATE PROTONATION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	37.10		26.80			-39.90	24.98	409.0
2	37.40		-40.00			53.60	24.98	409.2
3	99.71		-200.0	99.75		268.5	20.01	453.9

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.0	220.5	15.60	128.5	17.30	15.2	26.00	-86.
0.50	218.4	16.00	119.6	17.40	7.4	28.00	-95.
3.00	207.6	16.30	110.2	17.55	-1.5	30.00	-104.

Table 5 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
5.50	196.9	16.50	101.9	17.70	-8.3	32.00	-113.0
7.00	190.6	16.70	89.9	17.90	-15.3	34.00	-122.6
9.50	179.1	16.85	76.4	18.25	-24.6	36.00	-133.9
11.50	168.5	16.95	64.1	18.70	-33.2	37.50	-144.6
13.00	158.4	17.05	47.6	20.00	-49.8	38.50	-153.7
14.00	149.8	17.10	39.4	21.00	-58.4	39.30	-163.0
15.00	138.3	17.20	25.4	24.00	-76.9		
Titration No. 2							
1.30	-193.4	12.00	-85.9	18.50	56.9	21.50	159.0
1.50	-184.8	13.50	-75.6	18.60	72.7	23.00	172.2
1.90	-172.6	15.00	-63.1	18.70	85.5	25.00	185.8
2.50	-160.0	16.00	-52.2	18.80	94.9	27.00	197.5
3.00	-152.1	17.00	-36.4	18.95	105.5	29.00	208.6
4.00	-140.5	18.00	-2.7	19.15	115.3	30.00	214.4
5.00	-131.4	18.20	13.8	19.40	124.2	32.00	226.2
6.50	-120.1	18.30	25.1	19.80	134.4	34.00	239.0
8.00	-110.4	18.40	40.0	20.00	138.5	36.00	252.2
10.00	-98.4	18.45	48.2	20.50	146.8	38.00	263.8
Titration No. 3							
5.35	-160.7	8.50	-69.1	14.50	56.0	16.00	185.0
5.45	-148.3	9.50	-57.4	14.60	77.4	16.50	193.1
5.55	-139.4	10.00	-51.9	14.65	91.4	17.50	205.8
5.70	-129.8	11.00	-40.6	14.70	105.4	19.00	219.1
5.90	-120.0	12.00	-28.0	14.80	125.9	20.10	226.9
6.20	-109.4	13.00	-11.8	14.90	138.7	22.10	238.1
6.50	-101.3	13.50	-0.5	15.00	147.4	24.10	249.1
7.00	-91.0	14.00	16.0	15.20	159.6	27.10	264.1

Table 5 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
7.50	-82.6	14.30	33.4	15.50	171.4	30.10	281.1

Table 6 EXPERIMENTAL DATA FOR GLYCYLGLYCYLGLYCINATE PROTONATION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	40.70		-40.00			53.60	19.99	454.3
2	40.30		53.70			-39.90	24.98	452.8
3	40.30		53.70	40.10		-40.00	19.99	454.4

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.80	-157.5	7.50	-56.9	14.60	68.8	16.30	180.1
1.00	-145.7	9.00	-45.4	14.70	83.2	17.20	194.4
1.40	-129.7	10.50	-32.5	14.80	99.4	18.50	208.8
1.80	-119.0	12.00	-16.8	14.90	114.8	20.00	221.8
2.40	-107.0	13.00	-2.1	15.00	126.9	22.00	236.6
3.40	-93.1	14.00	23.9	15.15	139.9	24.00	251.0
4.50	-81.5	14.25	36.6	15.35	151.7		
6.00	-68.5	14.45	52.0	15.70	165.3		
Titration No. 2							
0.50	338.8	22.00	232.5	33.80	69.2	50.00	-72.6
2.00	333.7	24.00	224.3	34.10	49.3	52.00	-83.6
4.00	325.9	26.00	215.2	34.60	30.7	54.00	-96.8
6.00	317.8	28.00	204.3	35.40	14.5	56.00	-116.9

Table 6 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont,							
8.00	306.9	30.00	189.9	36.00	6.1	58.00	-158.7
10.00	294.0	31.00	179.9	37.00	-4.5	58.40	-170.3
12.00	280.5	32.00	165.2	39.00	-19.5	58.80	-180.5
14.00	268.2	32.50	154.0	40.00	-25.5	59.40	-192.3
16.00	258.1	33.00	136.1	42.00	-35.9	60.00	-200.6
18.00	249.0	33.30	117.4	44.00	-45.3		
20.00	240.6	33.50	98.9	46.00	-54.1		
Titration No. 3							
1.00	338.3	12.00	232.8	26.30	127.8	30.00	18.3
2.50	323.5	14.00	223.4	26.70	110.0	31.50	7.6
3.50	310.0	16.00	214.5	27.00	92.0	33.20	-0.8
4.50	294.4	18.50	203.0	27.20	79.5	35.00	-7.6
6.00	274.2	20.00	195.6	27.50	64.3	36.00	-10.8
7.00	264.1	22.50	180.4	27.90	50.3		
8.50	252.5	24.00	168.0	28.50	36.9		
10.00	243.2	25.40	149.8	29.50	23.2		

APPENDIX 2 : EXPERIMENTAL DATA FOR LIGAND-METAL COMPLEX FORMATION CONSTANT DETERMINATION

Table 1 EXPERIMENTAL DATA FOR LEAD-ASPARTATE INTERACTION

Titn. No.	Starting Solution A mM	Starting Solution E mM	Starting Solution H mM	Titrating Solution A mM	Titrating Solution E mM	Titrating Solution H mM	Initial Vol. ml	E° mV
1	19.94	4.909	9.016			-40.07	20.01	438.0
2	9.953	4.909	9.016			-40.07	20.01	438.0
3	5.142	4.909	9.016			-40.07	20.01	438.4
4	20.21	2.456	4.510			-40.07	20.01	438.7
5	10.16	2.456	4.510			-40.07	20.01	438.8
6	2.570	4.907	9.012			-40.07	20.01	438.5

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	287.4	11.60	185.4	16.20	67.4	17.23	-39.8
1.00	281.2	12.40	175.2	16.40	58.0	17.27	-46.5
2.00	273.9	13.50	156.6	16.60	46.9	17.35	-56.7
3.00	265.9	13.90	147.6	16.70	40.3	17.40	-61.7
5.00	248.3	14.30	136.4	16.90	22.1	17.45	-66.0
6.00	239.0	14.60	126.7	16.95	15.9	17.55	-73.0
7.00	229.8	14.80	119.8	17.00	8.7	17.70	-80.8
7.90	221.6	15.20	105.5	17.05	-0.8	17.90	-88.7
9.50	206.9	15.40	98.1	17.13	-18.7	18.10	-95.1
10.00	202.1	15.60	90.9	17.16	-25.5	18.40	-103.3
10.80	194.2	15.80	83.5	17.20	-34.0		
Titration No. 2							
0.10	300.2	7.30	202.8	10.20	102.7	11.70	26.8
1.00	292.7	8.30	181.8	10.30	97.8	11.80	17.6
2.00	283.1	8.70	171.1	10.40	93.3	11.85	11.9
2.80	274.2	9.00	161.3	10.50	88.8	12.00	-10.1
3.50	265.2	9.55	136.6	10.80	75.7	12.05	-19.5
4.90	248.0	9.70	128.6	11.00	66.8	12.09	-26.3
5.50	234.0	9.80	123.2	11.15	59.8		

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
6.10	223.9	9.90	117.9	11.30	52.2		
6.70	213.6	10.10	107.6	11.60	34.3		
Titration No. 3							
0.10	309.0	5.30	224.7	7.30	128.4	8.60	51.0
1.00	301.3	5.90	206.0	7.50	111.9	8.75	44.3
2.00	291.0	6.20	195.4	7.60	104.8	9.00	32.7
2.80	280.7	6.45	185.3	7.70	98.4	9.10	27.6
3.40	271.1	6.70	173.3	7.85	89.3	9.30	16.9
4.30	252.2	7.00	154.0	8.15	73.1	9.50	4.5
4.70	241.9	7.10	145.8	8.30	65.5	9.60	-2.2
5.00	233.5	7.20	137.3	8.45	58.1		
Titration No. 4							
0.10	270.0	11.70	141.2	13.50	23.8	13.89	-62.3
1.00	262.5	12.00	131.0	13.54	13.8	14.05	-74.1
2.00	253.4	12.50	108.0	13.60	-5.9	14.15	-79.6
3.50	239.2	12.70	97.0	13.63	-16.5	14.30	-86.3
6.00	216.1	12.85	88.0	13.65	-23.3	14.50	-93.5
7.20	205.1	13.00	78.4	13.67	-28.4	15.10	-108.5
8.40	193.5	13.15	67.2	13.74	-43.6	15.50	-116.1
10.60	165.8	13.40	41.2	13.78	-49.9		
11.20	154.2	13.45	33.4	13.83	-56.2		
Titration No. 5							
0.10	279.4	6.80	156.1	8.10	70.5	8.66	-23.5
1.00	268.1	7.00	147.1	8.20	62.2	8.68	-31.4
1.70	257.8	7.20	135.9	8.30	52.8	8.72	-44.7
2.40	246.4	7.30	129.5	8.40	41.4	8.74	-50.0
3.10	234.5	7.50	115.3	8.45	34.7	8.77	-56.4

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont.							
4.30	213.7	7.60	107.9	8.50	26.2	8.80 →	-61.4
4.90	203.3	7.70	100.5	8.55	15.4	8.90	-72.9
5.50	191.7	7.80	93.3	8.61	-3.1		
6.00	180.7	8.00	78.5	8.63	-10.8		
Titration No. 6							
0.10	313.9	4.80	227.6	5.85	142.0	6.22	94.8
1.00	306.5	5.25	202.3	5.90	134.3	6.40	79.9
2.80	285.6	5.40	191.9	5.95	126.6	6.55	70.3
3.40	274.7	5.55	179.4	6.05	113.0	6.85	56.8
4.30	249.5	5.65	169.4	6.10	107.0	7.00	51.6
4.60	237.0	5.80	149.8	6.15	101.7		

Table 2 EXPERIMENTAL DATA FOR LEAD-CYSTEINATE INTERACTION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	7.994	0.981	1.802			-20.02	20.01	448.6
2	4.889	0.981	1.802			-20.02	20.01	452.8
3	2.031	0.980	1.802			-10.01	20.01	452.7

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	263.4	3.67	143.2	3.97	53.1	5.90	-30.5
1.00	245.3	3.69	138.7	4.01	47.5	6.30	-38.1
1.90	218.9	3.71	133.3	4.05	42.8	6.80	-46.5
2.40	204.7	3.73	126.5	4.10	37.6	7.30	-53.9

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
2.60	199.3	3.75	118.3	4.17	31.4	7.80	-61.2
2.80	193.8	3.77	109.2	4.25	25.4	8.30	-68.4
2.95	189.2	3.79	100.0	4.35	18.9	8.80	-75.6
3.10	184.1	3.81	91.9	4.45	13.2	9.40	-84.4
3.25	178.2	3.83	84.5	4.60	6.2	10.00	-93.7
3.35	173.4	3.85	78.0	4.75	0.2	10.60	-103.7
3.45	167.5	3.87	72.4	4.90	-5.0	11.20	-113.8
3.55	159.6	3.89	67.5	5.10	-11.3	11.80	-125.0
3.60	154.3	3.91	63.4	5.35	-18.2		
3.65	147.1	3.94	57.7	5.60	-24.2		
Titration No. 2							
0.10	273.0	2.01	214.6	3.70	115.6	7.90	-106.1
0.30	269.6	2.12	210.8	3.75	88.5	8.10	-111.9
0.50	265.6	2.27	206.0	3.80	66.3	8.30	-117.9
0.70	261.2	2.42	201.4	3.85	52.6	8.50	-124.0
0.90	256.2	2.60	196.1	3.95	35.5	8.70	-129.9
1.10	250.1	2.80	190.3	4.15	14.8	8.90	-136.0
1.25	244.9	3.00	183.9	4.35	1.2	9.10	-141.8
1.35	241.0	3.10	180.3	4.80	-19.6	9.30	-147.6
1.45	236.9	3.20	176.1	5.40	-39.5	9.50	-153.1
1.55	232.7	3.30	171.6	6.10	-58.4	9.70	-158.2
1.65	228.5	3.40	165.9	6.80	-76.5		
1.77	223.6	3.50	158.3	7.50	-94.9		
1.89	218.9	3.65	135.7	7.70	-100.4		
Titration No. 3							
1.40	270.3	3.40	224.6	5.30	182.9	7.80	26.0
1.80	264.7	3.55	219.5	5.80	174.8	7.90	11.6

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
2.10	259.7	3.70	214.9	6.40	164.0	8.00	0.9
2.30	255.9	3.90	209.6	6.80	154.9	8.20	-14.8
2.55	250.4	4.10	204.8	7.30	135.2	8.50	-32.1
2.75	245.4	4.30	200.4	7.50	112.6	8.90	-50.4
2.95	239.7	4.50	196.5	7.60	82.4	9.40	-70.5
3.10	234.9	4.70	192.9	7.65	63.6		
3.25	229.8	4.90	189.5	7.70	48.1		

Table 3 EXPERIMENTAL DATA FOR ZINC-CYSTEINATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	4.658	2.354	4.722			-20.00	20.01	453.4
2	9.448	2.354	4.722			-20.00	20.01	453.8
3	14.11	2.354	4.722			-50.04	20.01	453.4
4	2.379	2.356	4.726			-20.00	20.01	453.6
5	9.415	4.711	9.450			-50.04	20.01	452.8
6	4.724	2.354	4.722			-20.02	20.01	448.8
7	11.76	2.354	4.722			-20.02	20.01	450.0

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
4.65	187.3	4.90	146.3	7.50	103.8	12.70	29.9
4.68	179.5	5.00	140.3	8.50	94.8	13.10	17.7
4.70	174.4	5.15	134.1	9.50	84.7	13.40	3.8
4.72	169.5	5.30	129.8	10.00	78.9	13.60	-12.7

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont,							
4.74	165.3	5.50	125.5	10.70	69.3	13.82	-61.7
4.77	160.3	5.90	119.5	11.30	59.6	13.90	-80.9
4.80	156.0	6.40	113.9	11.90	48.3		
4.85	150.5	6.90	109.2	12.30	39.8		
Titration No, 2							
4.56	200.8	6.00	133.5	13.60	39.1	16.90	-65.3
4.60	195.6	7.00	123.6	14.00	24.9	17.40	-75.8
4.65	188.7	8.00	115.6	14.30	9.9	17.80	-84.7
4.72	178.6	9.50	102.9	14.50	-0.4	18.20	-94.5
4.78	171.8	10.50	92.3	14.80	-13.2	18.60	-105.3
4.85	165.3	11.30	81.5	15.10	-23.5	19.00	-116.7
4.95	158.6	12.00	70.4	15.50	-34.9	19.30	-125.3
5.15	149.9	12.60	60.2	15.90	-44.3	19.60	-133.4
5.40	143.2	13.10	50.9	16.40	-55.1	20.00	-143.1
Titration No, 3							
1.80	201.6	3.30	125.0	5.65	23.8	8.00	-71.3
1.83	194.2	3.70	116.3	5.75	14.4	8.40	-82.6
1.86	187.2	4.10	105.2	5.85	6.2	8.75	-93.4
1.90	179.0	4.40	93.8	5.95	-1.1	9.05	-103.6
1.95	171.1	4.60	84.8	6.10	-10.5	9.30	-112.9
2.00	165.4	4.80	75.7	6.30	-20.5	9.55	-122.5
2.10	157.4	5.00	66.3	6.55	-30.4	9.85	-133.3
2.25	149.9	5.20	56.4	6.90	-41.7		
2.50	141.8	5.40	44.4	7.20	-50.2		
2.80	135.0	5.55	32.9	7.60	-60.9		

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 4							
4.73	153.1	4.90	131.1	5.85	105.5	8.05	75.4
4.76	146.7	5.05	123.0	6.60	95.8	8.50	65.8
4.80	140.7	5.35	114.1	7.40	85.5		
Titration No. 5							
3.74	183.8	3.82	169.4	4.15	147.8		
3.78	175.6	3.95	157.5	4.45	139.6		
Titration No. 6							
4.70	155.9	5.50	120.6	10.00	75.1	12.80	24.6
4.72	153.0	5.80	116.3	10.40	69.9	13.00	19.1
4.75	149.4	6.20	111.8	10.80	64.1	13.15	14.2
4.78	146.3	6.60	107.8	11.10	59.3	13.35	6.4
4.82	143.0	7.10	103.3	11.30	55.9	13.50	-1.2
4.89	138.4	7.60	99.0	11.60	50.4	13.60	-7.7
4.97	134.5	8.10	94.7	11.85	45.7	13.70	-16.4
5.07	130.6	8.60	90.1	12.10	40.7	13.75	-22.5
5.18	127.3	9.10	85.2	12.35	35.3	13.80	-30.5
5.30	124.4	9.50	80.9	12.56	30.5		
Titration No, 7							
4.50	195.7	7.45	120.8	13.30	45.3	15.70	-30.4
4.55	190.1	8.10	115.6	13.50	40.3	16.00	-36.0
4.59	185.4	8.70	110.7	13.70	34.6	16.25	-40.4
4.64	179.7	9.30	105.6	13.85	29.7	16.55	-45.2
4.68	175.5	9.80	100.7	14.00	24.2	16.90	-50.5
4.73	170.7	10.00	98.6	14.10	20.0	17.20	-54.8
4.78	166.6	10.70	90.1	14.20	15.8	17.60	-60.5
4.87	161.0	11.00	85.9	14.35	9.3	17.95	-65.2
4.98	155.7	11.30	81.3	14.45	5.1	18.35	-70.8

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 7 cont.							
5.12	150.7	11.70	74.7	14.60	-0.7	18.65	-75.1
5.30	145.8	11.90	71.4	14.70	-4.3	19.00	-80.1
5.55	140.8	12.30	64.5	14.90	-10.8	19.35	-85.4
5.90	135.6	12.60	59.2	15.10	-16.6	19.65	-90.0
6.30	131.0	12.80	55.6	15.25	-20.4	20.00	-95.8
6.85	125.8	13.05	50.7	15.45	-25.1		

Table 4 EXPERIMENTAL DATA FOR LEAD-GLUTAMATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	19.97	9.805	18.01			-40.05	20.01	450.9
2	9.986	4.903	9.006			-40.05	20.01	450.2
3	20.00	4.900	9.000			-40.05	20.01	450.2
4	0. 00	2.450	4.501			-40.05	20.01	449.8
5	19.99	2.450	4.501			-40.05	20.01	449.8

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
1.00	313.2	9.50	243.4	17.00	160.1	20.00	70.2
2.00	306.9	10.00	237.7	17.70	147.8	20.40	63.0
3.50	296.9	11.00	226.6	18.20	136.3	21.00	54.2
4.50	289.8	12.00	215.9	18.70	119.7	21.80	43.3
5.50	282.1	13.00	205.6	19.00	106.6	22.60	31.5
6.50	273.9	14.00	195.4	19.20	96.6	23.20	21.7
7.50	264.5	15.00	185.0	19.50	83.6	23.80	11.0

Table 4 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
8.50	254.3	16.00	173.7	19.80	74.7	24.40	-1.0
Titration No. 2							
0.10	306.0	4.60	240.6	8.20	164.3	9.65	83.4
0.70	300.5	5.10	229.7	8.60	152.4	9.75	76.1
1.40	292.8	5.50	221.0	8.90	141.0	9.90	68.4
2.00	285.6	5.90	212.7	9.10	130.8	10.00	64.4
2.60	277.4	6.30	204.7	9.25	120.8	10.40	52.2
3.20	268.2	6.80	194.9	9.35	112.6	10.80	41.7
3.70	259.2	7.30	184.8	9.45	102.8	11.20	30.8
4.20	249.3	7.80	174.1	9.55	92.7	11.60	18.8
Titration No. 3							
0.10	287.9	5.60	231.4	11.80	163.9	14.65	80.9
0.50	284.7	6.30	223.2	12.50	153.8	14.85	68.6
1.00	280.6	7.10	214.2	13.10	143.2	15.10	56.6
2.00	271.7	8.00	204.7	13.60	131.4	15.40	46.0
3.00	261.7	9.00	194.5	14.00	118.2	15.75	36.0
4.00	250.6	10.00	184.3	14.30	103.8	16.10	26.1
4.80	241.1	10.90	174.7	14.50	91.1		
Titration No. 4							
0.20	278.5	4.40	192.9	6.90	120.6	7.40	62.3
1.10	264.1	4.90	183.1	7.00	112.4	7.50	53.2
1.80	249.9	5.40	172.6	7.10	101.9	7.65	43.0
2.40	236.1	5.80	163.3	7.20	89.1	7.85	32.4
2.80	226.7	6.20	152.2	7.25	81.6	8.05	21.7
3.30	215.5	6.50	141.5	7.30	74.4	8.25	10.3
3.90	203.0	6.70	132.4	7.35	67.9	8.50	-6.0

Table 4 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5							
0.20	265.7	6.40	193.9	11.50	121.4	12.55	47.5
1.10	255.7	7.40	183.9	11.75	111.3	12.65	40.2
2.00	244.8	8.40	173.6	11.90	103.4	12.80	30.6
2.80	234.6	9.30	163.3	12.10	89.1	12.95	21.7
3.60	224.5	10.00	153.9	12.20	80.3	13.10	12.9
4.50	213.9	10.60	143.9	12.35	65.5	13.30	0.7
5.50	203.2	11.10	133.3	12.45	56.0		

Table 5 EXPERIMENTAL DATA FOR LEAD-ETHYLENEDIAMINETETRAACETATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	29.91	4.906	-31.05			311.4	20.01	438.7
2	19.99	4.906	-31.05			311.4	20.01	439.1
3	10.00	2.455	-15.54			155.9	20.01	439.2
4	9.969	4.906	-11.03			155.9	20.01	439.3
5	4.976	2.453	-15.55			77.96	20.01	439.2
6	5.019	4.906	-11.04			77.96	20.01	433.0
7	10.00	4.906	-11.04			77.96	20.01	433.1

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	-1.5	1.25	93.1	1.43	196.8	2.55	276.0
0.20	5.5	1.30	108.6	1.45	203.2	2.80	282.0
0.30	12.2	1.33	123.4	1.48	210.8	3.10	288.2

Table 5 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont,							
0.40	18.5	1.34	130.6	1.52	218.7	3.60	297.1
0.55	27.6	1.35	139.9	1.57	226.2	4.10	304.6
0.70	36.8	1.36	149.3	1.63	233.0	4.60	311.4
0.85	46.8	1.37	158.4	1.70	239.3	5.10	317.6
1.00	58.7	1.38	167.5	1.80	246.5	5.60	323.3
1.10	69.0	1.39	175.4	1.95	254.9	6.10	328.5
1.15	75.4	1.40	182.7	2.10	261.6	6.70	334.3
1.20	83.0	1.41	187.7	2.30	268.7	7.30	339.6
Titration No. 2							
0.10	-73.3	0.90	30.8	1.41	191.5	2.40	281.6
0.20	-61.0	1.00	41.3	1.40	186.5	3.10	299.7
0.30	-46.5	1.10	53.6	1.43	200.2	2.70	290.3
0.35	-38.2	1.20	69.3	1.49	218.4	3.60	309.5
0.40	-29.7	1.25	80.2	1.46	210.4	4.60	325.2
0.45	-21.4	1.28	89.1	1.54	228.0	4.10	317.9
0.50	-14.2	1.31	101.4	1.70	246.5	5.10	331.6
0.55	-7.6	1.34	123.3	1.60	236.2	6.10	342.1
0.62	1.1	1.36	145.3	1.81	255.1	5.60	337.2
0.70	10.2	1.38	169.2	2.15	272.6	6.70	347.2
0.80	20.7	1.39	179.7	1.95	263.4	7.30	351.6
Titration No. 3							
0.10	-72.7	1.25	81.2	1.85	254.8	4.30	312.2
0.20	-60.2	1.30	97.7	1.90	257.7	4.70	316.7
0.25	-53.2	1.33	113.2	2.00	262.8	5.20	321.8
0.30	-45.6	1.37	153.6	2.10	267.2	5.70	326.2
0.35	-37.2	1.39	173.3	2.20	271.0	6.20	330.0
0.45	-20.8	1.41	187.6	2.35	276.1	6.80	334.1

Table 5 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
0.55	-6.3	1.43	198.4	2.50	280.5	7.50	338.2
0.60	0.1	1.45	205.9	2.70	285.5	8.30	342.1
0.70	11.4	1.50	218.9	2.90	290.0	9.10	345.5
0.80	21.7	1.55	227.7	3.10	294.0	10.00	348.8
0.90	31.8	1.65	239.7	3.35	298.6		
1.05	48.2	1.75	248.1	3.60	302.6		
1.20	70.3	1.80	251.6	3.90	306.9		
Titration No. 4							
0.10	112.8	0.22	206.4	0.95	269.4	3.20	315.7
0.11	124.4	0.24	211.5	1.05	273.1	3.60	320.2
0.12	137.1	0.28	219.7	1.20	278.1	4.10	325.1
0.13	150.1	0.33	227.7	1.35	282.5	4.60	329.4
0.14	162.9	0.40	236.1	1.50	286.6	5.20	333.8
0.15	172.4	0.47	242.7	1.70	291.2	6.00	338.7
0.16	180.1	0.55	248.8	1.90	295.5	6.80	342.7
0.17	186.4	0.65	255.2	2.20	301.1	7.80	347.0
0.18	191.4	0.75	260.5	2.50	306.1	9.00	351.1
0.20	200.0	0.85	265.2	2.80	310.5		
Titration No. 5							
1.80	-77.8	2.54	60.2	2.84	206.4	4.90	291.4
1.90	-60.2	2.60	74.8	2.90	217.7	5.20	295.5
1.95	-49.7	2.65	92.5	2.96	225.9	5.60	300.2
2.00	-37.9	2.67	102.4	3.05	234.9	6.00	304.2
2.05	-25.2	2.69	116.8	3.15	242.5	6.50	308.6
2.10	-13.5	2.70	125.7	3.25	248.5	7.20	313.7
2.15	-3.5	2.71	135.9	3.40	255.9	8.00	318.5
2.20	5.2	2.72	145.1	3.60	263.6	9.00	323.5

Table 5 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont.							
2.25	13.4	2.74	162.4	3.80	269.7	10.00	327.5
2.30	21.2	2.76	176.8	4.00	274.8	11.50	332.4
2.38	33.1	2.78	188.1	4.30	281.4		
2.46	45.5	2.80	195.1	4.60	286.8		
Titration No. 6							
0.44	213.4	1.40	264.3	5.50	314.3	18.00	342.7
0.52	221.9	1.80	274.3	7.00	321.3	20.00	344.6
0.62	230.3	2.30	283.9	8.50	326.5	25.00	348.1
0.75	238.6	2.90	292.7	10.00	330.5	30.00	350.8
0.90	246.3	3.60	300.5	12.00	334.6		
1.10	254.5	4.50	308.0	15.00	339.3		
Titration No. 7							
0.05	73.4	0.32	183.8	1.20	246.1	8.00	314.6
0.10	83.9	0.36	192.4	1.50	254.2	10.00	321.9
0.20	126.9	0.41	200.4	2.00	264.5	13.00	329.7
0.22	141.5	0.50	210.7	3.00	279.2	16.00	335.1
0.24	153.6	0.60	218.9	4.00	289.8	20.00	340.3
0.26	163.8	0.75	228.2	5.00	297.9	25.00	344.7
0.29	175.6	0.95	237.3	6.50	307.4	30.00	347.8

Table 6 EXPERIMENTAL DATA FOR ZINC-ETHYLENEDIAMINETETRAACETATE INTERACTION

Titn. No.	Starting Solution A mM	Starting Solution B mM	Starting Solution H mM	Titrating Solution A mM	Titrating Solution B mM	Titrating Solution H mM	Initial Vol. ml	E^o mV
1	7.967	3.997	8.175			-20.02	20.01	434.0
2	7.986	3.997	20.64			62.34	20.01	435.1
3	8.007	3.997	-11.84			124.6	20.01	435.2
4	4.007	2.000	-5.927			62.34	20.01	434.6
5	16.02	3.997	-31.88			311.4	20.01	435.7
6	3.987	3.997	-11.86			124.6	20.01	434.2
7	23.94	3.997	-31.88			311.4	20.01	434.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	303.5	10.00	262.5	15.10	201.1	16.00	124.9
0.30	302.9	10.50	259.3	15.20	197.7	16.02	121.1
0.70	301.6	11.00	255.8	15.25	195.7	16.05	115.5
1.50	299.0	11.40	252.8	15.30	193.7	16.08	110.4
2.00	297.4	11.80	249.6	15.35	191.6	16.11	105.7
3.00	294.0	12.20	246.1	15.40	189.2	16.14	101.4
4.00	290.4	12.50	243.3	15.45	186.6	16.17	97.8
4.50	288.5	12.80	240.3	15.55	180.8	16.30	84.6
5.00	286.7	13.10	237.0	15.60	177.4	16.50	71.1
5.50	284.7	13.40	233.4	15.70	169.2	16.80	57.4
6.00	282.6	13.70	229.5	15.75	164.5	17.20	43.9
6.50	280.5	13.90	226.7	15.78	161.0	17.70	30.2
7.00	278.3	14.10	223.6	15.81	157.2	18.30	15.2
7.50	276.0	14.30	220.1	15.87	148.6	19.00	-3.2
8.00	273.6	14.50	216.2	15.90	143.7	20.00	-38.8
8.50	271.0	14.70	211.9	15.93	138.3		
9.00	268.4	14.85	208.3	15.96	132.7		
9.50	265.5	15.00	204.3	15.98	128.9		

Table 6 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2							
0.10	325.9	3.00	332.7	8.50	340.4	18.00	347.5
0.20	326.2	4.00	334.5	10.00	341.9	20.00	348.5
0.40	326.8	5.00	336.0	12.00	343.6	22.00	349.4
1.00	328.4	6.00	337.4	14.00	345.1		
2.00	330.6	7.00	338.7	16.00	346.4		
Titration No. 3							
0.05	-20.9	0.54	70.3	0.74	198.1	2.15	283.2
0.06	-18.1	0.56	77.2	0.76	202.7	2.50	290.8
0.07	-15.9	0.58	86.8	0.79	208.7	2.90	297.8
0.08	-13.6	0.60	99.6	0.82	213.8	3.40	304.9
0.10	-9.3	0.61	107.4	0.86	219.3	4.00	311.6
0.12	-5.5	0.62	118.6	0.90	224.2	4.60	317.0
0.14	-1.7	0.63	132.2	0.95	229.5	5.30	322.1
0.16	1.8	0.64	146.1	1.02	235.7	6.20	327.4
0.20	8.6	0.65	155.0	1.10	241.8	7.20	332.1
0.25	16.3	0.66	164.5	1.18	247.1	8.50	337.0
0.30	23.9	0.67	170.8	1.28	252.8	10.00	341.4
0.35	31.7	0.68	177.2	1.40	258.6	12.00	346.2
0.40	39.7	0.69	181.9	1.55	265.0	14.00	349.9
0.45	48.6	0.70	185.9	1.70	270.4	17.00	354.4
0.50	59.3	0.72	192.4	1.90	276.6		
Titration No. 4							
0.02	-27.4	0.58	83.7	0.77	197.7	2.90	286.4
0.04	-22.0	0.60	93.6	0.79	201.9	3.20	290.3
0.06	-17.2	0.61	100.5	0.82	207.1	3.60	294.8
0.10	-8.8	0.62	107.8	0.85	211.6	4.10	299.5
0.13	-3.1	0.63	116.8	0.89	216.6	4.70	304.2

Table 6 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 4 cont.							
0.16	2.2	0.64	128.6	0.94	222.0	5.40	308.7
0.20	8.7	0.65	142.9	1.00	227.6	6.20	312.8
0.24	14.9	0.66	152.5	1.08	233.8	7.20	317.1
0.29	22.5	0.67	160.2	1.18	240.1	8.40	321.2
0.35	31.5	0.68	166.7	1.28	245.5	9.00	323.0
0.40	39.5	0.69	171.8	1.40	251.0	11.00	327.8
0.45	48.1	0.70	175.9	1.55	256.8	14.00	333.1
0.50	58.4	0.71	180.2	1.75	263.3	17.00	337.0
0.53	66.2	0.72	184.0	2.00	269.9	19.30	339.4
0.55	71.8	0.73	187.3	2.30	276.5		
0.57	79.2	0.75	193.0	2.60	281.8		
Titration No. 5							
0.65	-59.6	1.45	73.4	1.64	209.0	2.70	288.9
0.75	-40.2	1.50	92.4	1.67	216.6	3.00	297.3
0.80	-29.8	1.53	112.9	1.72	227.2	3.30	304.6
0.85	-20.0	1.55	140.8	1.77	234.6	3.70	312.7
0.90	-11.1	1.56	156.5	1.85	244.2	4.10	319.7
0.97	-0.2	1.57	168.8	1.95	253.3	4.50	325.6
1.05	10.9	1.58	179.0	2.05	260.5	4.90	330.7
1.15	23.5	1.59	186.5	2.15	266.5	5.30	335.1
1.25	36.5	1.60	193.0	2.30	274.0	5.70	339.0
1.35	51.8	1.62	201.8	2.50	282.1	6.20	343.1
Titration No. 6							
0.63	80.5	0.77	208.2	1.40	268.4	3.80	317.4
0.64	115.2	0.80	214.4	1.50	273.3	4.30	321.5
0.65	141.1	0.84	221.3	1.65	279.5	5.00	326.1
0.66	157.5	0.88	226.8	1.80	284.7	5.80	330.4

Table 6 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 6 cont.							
0.67	167.2	0.95	235.3	2.00	290.5	6.80	334.9
0.68	175.4	1.00	240.3	2.20	295.4	8.00	339.3
0.69	181.7	1.07	246.6	2.40	299.5	9.20	342.8
0.70	186.6	1.14	252.2	2.70	304.6	10.00	344.9
0.72	194.7	1.22	257.8	3.00	308.8	12.00	349.2
0.74	200.9	1.30	262.9	3.40	313.5		
Titration No. 7							
0.10	-58.2	1.40	73.1	1.64	201.1	2.45	268.5
0.18	-48.7	1.45	83.8	1.66	206.5	2.60	273.4
0.25	-39.7	1.50	100.4	1.69	213.0	2.80	279.2
0.33	-29.6	1.52	110.7	1.72	218.2	3.00	284.3
0.42	-19.0	1.54	125.6	1.76	224.2	3.30	291.0
0.50	-10.6	1.55	136.4	1.80	229.1	3.60	296.8
0.60	-1.3	1.56	147.5	1.85	234.6	4.00	303.7
0.70	7.0	1.57	160.2	1.93	241.7	4.40	309.9
0.85	18.5	1.58	168.7	2.01	247.5	4.80	315.5
1.00	30.0	1.59	179.8	2.10	252.9	5.20	320.7
1.15	42.7	1.60	185.0	2.20	258.1	5.60	325.3
1.30	58.2	1.62	193.5	2.30	262.7	6.10	330.6

Table 7 EXPERIMENTAL DATA FOR LEAD-D-PENICILLAMINATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	9.904	2.457	4.513			-50.14	20.01	433.9
2	20.05	4.912	9.022			-50.14	20.01	434.1
3	9.958	4.912	9.022			-50.14	20.01	435.2
4	5.153	4.912	9.022			-50.14	20.01	434.9
5	30.01	4.912	9.022			-50.14	20.01	434.7
6	5.100	2.457	4.513			-50.14	20.01	435.5
7	15.00	2.457	4.513			-50.14	20.01	434.9

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	270.0	3.79	126.8	4.10	-5.5	7.50	-146.7
0.70	257.9	3.81	103.4	4.20	-14.7	8.10	-178.3
1.60	234.4	3.82	90.4	4.50	-33.4	8.40	-192.2
1.90	226.7	3.84	66.9	4.70	-42.4	9.00	-213.6
2.50	212.4	3.85	58.5	5.20	-60.6	9.30	-221.8
2.90	202.7	3.88	39.8	5.50	-70.3	10.00	-237.6
3.60	174.1	3.90	31.6	6.30	-96.0		
3.70	162.1	3.97	13.2	6.60	-106.8		
3.77	141.3	4.01	6.2	7.20	-131.8		
Titration No. 2							
0.10	279.2	6.00	208.8	7.82	39.5	13.10	-98.0
1.00	270.0	6.80	195.4	7.87	30.5	14.70	-130.1
2.00	258.1	7.20	184.2	8.05	11.1	15.30	-145.3
2.40	252.9	7.50	165.8	8.20	1.1	16.30	-173.1
3.20	242.4	7.60	148.8	8.70	-19.6	16.80	-185.6
3.60	237.4	7.67	114.1	9.10	-30.5	17.80	-205.9
4.40	227.8	7.69	96.3	10.00	-48.8	19.10	-225.9

Table 7 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
4.80	223.3	7.73	67.8	10.70	-60.4		
5.60	213.9	7.75	59.0	12.30	-85.0		
Titration No. 3							
0.10	295.2	5.20	213.2	7.74	106.0	8.35	-36.0
1.00	284.9	5.50	209.0	7.76	82.5	8.50	-44.4
1.50	278.1	6.10	200.2	7.77	71.6	9.00	-67.0
2.50	261.2	6.40	195.3	7.79	52.7	9.30	-79.3
2.80	255.1	7.00	183.0	7.81	39.8	10.00	-108.4
3.40	242.5	7.30	173.5	7.86	19.6	10.30	-121.4
3.70	236.6	7.60	153.4	7.90	9.5	10.90	-148.8
4.30	226.3	7.65	145.5	8.00	-6.6	11.10	-158.8
4.60	221.7	7.72	121.4	8.10	-17.6	11.50	-180.0
Titration No. 4							
0.10	304.6	3.00	256.5	4.80	209.8	7.00	159.0
0.50	300.5	3.40	243.5	5.20	202.5	7.30	142.5
1.00	294.7	3.70	234.2	5.70	193.3	7.50	122.4
1.70	284.9	4.00	226.4	6.20	182.9	7.55	113.7
2.40	272.0	4.40	217.4	6.60	172.7	7.60	100.3
Titration No. 5							
0.10	269.7	6.00	211.6	18.50	-126.2	21.50	-179.6
1.00	262.1	6.70	200.4	19.00	-134.5	22.50	-193.1
2.00	252.5	7.20	186.4	19.50	-143.7	23.50	-204.4
3.00	242.7	16.00	-93.8	20.00	-153.2		
4.00	232.9	17.00	-105.2	20.50	-162.7		
5.00	223.0	18.00	-118.5	21.00	-171.6		

Table 7 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 6							
0.10	282.2	3.20	188.3	3.90	14.6	5.00	-112.0
0.50	274.2	3.40	180.3	3.92	5.7	5.15	-125.1
1.20	254.3	3.75	151.1	3.98	-11.7	5.45	-151.8
1.40	246.7	3.80	134.1	4.02	-19.7	5.60	-165.0
1.80	230.8	3.83	101.7	4.20	-43.5	5.95	-194.6
2.00	223.7	3.84	80.4	4.30	-53.4		
2.40	211.5	3.86	47.3	4.55	-74.7		
2.60	206.0	3.87	35.6	4.70	-86.7		
Titration No. 7							
0.10	263.1	3.83	128.9	4.05	21.6	6.50	-65.0
1.00	246.9	3.85	108.4	4.12	12.9	7.70	-87.7
2.00	226.8	3.86	96.8	4.30	-2.3	8.40	-102.7
2.50	216.9	3.88	77.0	4.45	-11.0	9.50	-133.0
3.40	192.7	3.89	68.5	4.80	-25.2	10.00	-150.3
3.70	171.4	3.92	52.7	5.10	-34.5		
3.80	147.9	3.94	45.3	6.00	-55.2		

Table 8 EXPERIMENTAL DATA FOR LEAD-GLUTATHIONATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	19.99	2.452	4.504			-50.04	20.01	451.3
2	3.933	0.981	1.802			-50.04	20.01	450.5
3	6.004	0.981	1.802			-50.04	20.01	449.1

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.20	276.3	9.50	181.4	11.50	76.7	12.95	-19.4
0.50	273.8	10.00	169.8	11.55	71.5	13.15	-28.3
1.00	269.7	10.20	163.8	11.60	66.8	13.40	-37.5
2.00	261.0	10.40	156.8	11.70	57.6	13.70	-46.3
3.00	252.2	10.60	148.4	11.80	49.3	14.00	-53.4
4.00	243.3	10.80	138.0	11.90	41.5	14.40	-61.3
5.00	234.2	10.90	131.8	12.00	34.3	14.90	-69.1
6.00	225.0	11.00	124.7	12.10	27.3	15.50	-77.1
7.00	215.2	11.10	116.5	12.20	20.9	16.20	-84.9
7.50	209.9	11.20	107.4	12.30	14.9	17.20	-94.4
8.00	204.3	11.30	97.5	12.45	6.3	18.20	-103.1
8.50	197.9	11.40	87.0	12.60	-1.8		
9.00	190.5	11.45	81.8	12.75	-9.6		
Titration No. 2							
0.10	274.9	2.10	188.2	2.83	86.8	3.29	-22.0
0.20	272.0	2.20	181.0	2.85	80.8	3.35	-30.5
0.40	265.9	2.30	172.9	2.88	71.9	3.40	-37.3
0.50	262.6	2.40	163.6	2.90	65.8	3.45	-43.7
0.70	255.8	2.50	152.6	2.93	56.8	3.50	-49.4
0.90	248.5	2.55	145.8	2.95	50.7	3.60	-59.7
1.00	244.6	2.60	138.2	2.98	42.0	3.70	-68.6

Table 8 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont,							
1.20	236.4	2.65	129.7	3.01	33.8	3.80	-76.4
1.40	227.6	2.70	119.4	3.04	25.9	3.95	-86.6
1.55	220.4	2.72	114.7	3.10	12.0	4.10	-95.5
1.70	212.9	2.75	107.6	3.15	1.9	4.30	-106.2
1.85	204.4	2.78	100.2	3.19	-5.6		
2.00	195.1	2.80	95.2	3.24	-14.0		
Titration No. 3							
0.10	270.5	3.10	169.2	3.72	73.4	4.13	-7.6
0.40	263.5	3.20	161.7	3.74	67.8	4.20	-16.8
0.70	256.2	3.30	152.5	3.76	62.3	4.27	-25.2
1.00	248.3	3.35	147.2	3.78	56.8	4.34	-33.1
1.30	240.0	3.40	140.8	3.80	51.7	4.42	-41.2
1.55	232.6	3.45	133.4	3.82	46.9	4.50	-48.3
1.80	225.1	3.50	125.8	3.85	40.0	4.60	-55.9
2.05	216.9	3.54	117.8	3.88	33.6	4.70	-62.5
2.30	207.8	3.58	109.7	3.91	27.5	4.80	-68.1
2.50	199.8	3.61	102.7	3.95	19.9	4.95	-75.6
2.70	191.1	3.64	95.3	3.99	12.9	5.10	-82.2
2.85	184.0	3.67	87.6	4.03	6.4	5.30	-89.7
3.00	175.8	3.70	79.2	4.08	-1.0		

Table 9 EXPERIMENTAL DATA FOR ZINC-GLUTATHIONATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	4.702	2.354	4.722			-50.04	20.01	451.2
2	2.370	2.354	4.722			-50.04	20.01	451.2
3	1.178	2.354	4.722			-50.04	20.01	451.2
4	9.482	4.711	9.450			-50.04	20.01	451.8
5	2.362	1.176	2.360			-50.04	20.01	451.8

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
1.60	263.4	3.60	166.3	4.45	88.3	6.15	-9.1
1.90	254.4	3.65	158.9	4.60	81.9	6.20	-15.1
2.10	247.9	3.68	153.8	4.80	73.7	6.25	-21.3
2.30	240.9	3.71	148.6	5.00	65.2	6.30	-28.0
2.50	233.5	3.74	143.6	5.20	56.0	6.35	-34.6
2.70	225.8	3.78	137.4	5.35	48.5	6.42	-44.2
2.90	217.5	3.83	130.6	5.50	40.4	6.49	-53.1
3.10	207.6	3.88	124.5	5.65	31.6	6.56	-61.8
3.20	201.9	3.95	117.6	5.80	21.9	6.65	-71.7
3.30	195.5	4.05	109.7	5.90	14.7	6.75	-81.8
3.40	187.7	4.15	103.3	6.00	6.3		
3.50	178.3	4.30	95.3	6.10	-3.7		
Titration No. 2							
2.78	154.2	2.94	113.9	3.60	64.4	4.50	5.1
2.80	148.2	2.98	108.4	3.75	56.6	4.55	-0.5
2.82	142.0	3.05	100.3	3.90	48.8	4.60	-6.8
2.84	135.9	3.12	93.9	4.05	40.2	4.65	-13.4
2.86	130.5	3.20	87.8	4.20	30.7		
2.88	125.6	3.30	81.1	4.30	23.4		

Table 9 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
2.90	121.3	3.45	72.4	4.40	15.0		
Titration No. 3							
2.35	133.5	2.44	101.8	2.65	73.2	3.05	36.9
2.36	127.3	2.48	94.3	2.75	64.1	3.15	25.3
2.37	123.1	2.53	86.8	2.85	55.3		
2.38	119.1	2.58	80.6	2.95	46.6		
Titration No. 4							
3.50	263.6	7.25	170.8	9.80	83.6	12.50	-11.6
4.00	255.2	7.40	161.3	10.00	78.6	12.65	-21.2
4.50	246.2	7.55	151.5	10.40	67.9	12.80	-31.3
5.00	236.9	7.65	145.4	10.70	59.3	12.90	-38.2
5.40	228.9	7.80	137.3	11.00	50.0	13.00	-45.0
5.80	220.3	7.95	130.7	11.30	39.9	13.15	-55.0
6.10	213.2	8.10	125.2	11.50	32.9	13.30	-64.3
6.40	205.1	8.30	119.1	11.70	25.9	13.45	-72.8
6.70	195.6	8.60	111.3	11.90	18.2	13.60	-80.3
6.90	188.2	9.00	102.2	12.10	9.7	13.80	-89.2
7.10	179.0	9.40	93.1	12.30	-0.1	14.00	-97.4
Titration No. 5							
0.80	258.5	1.80	167.2	2.05	95.3	2.95	10.4
0.95	250.6	1.82	160.8	2.10	89.0	3.00	2.7
1.10	242.0	1.84	153.7	2.20	79.0	3.05	-6.8
1.25	232.3	1.86	145.8	2.30	70.5	3.10	-17.4
1.35	225.0	1.88	136.7	2.40	62.6	3.15	-28.8
1.45	216.7	1.90	128.7	2.50	54.9	3.20	-39.3
1.55	207.2	1.92	121.7	2.60	47.1	3.25	-50.7
1.65	195.2	1.94	115.9	2.70	38.6	3.30	-61.8

Table 9 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont.							
1.70	188.3	1.97	103.9	2.80	29.1	3.35	-71.8
1.75	179.5	2.00	103.3	2.90	17.7		

Table 10 EXPERIMENTAL DATA FOR LEAD-GLYCINATE INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	19.98	4.903	8.706			-10.02	20.01	453.7
2	40.10	9.813	17.42			-10.01	20.01	452.7
3	12.33	6.128	0.873			-10.01	20.01	452.7
4	14.80	7.361	13.07			-10.01	20.01	454.2
5	12.31	6.128	10.87			-10.01	20.01	455.5
6	40.13	4.906	8.711			-10.01	20.01	454.0
7	5.036	4.903	8.706			-20.01	20.01	457.5
8	2.560	4.906	8.711			-20.01	20.01	459.5
9	74.66	4.911	9.021			-20.04	20.01	458.2

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
10.00	251.0	18.00	174.6	19.30	102.2	25.00	44.7
11.50	244.9	18.20	164.3	19.50	96.5	26.20	36.0
13.00	237.8	18.35	154.9	19.80	89.8	27.40	26.7
14.50	228.7	18.45	147.9	20.00	86.2	28.60	16.6
15.50	220.6	18.60	137.1	20.40	80.4	29.50	7.9
16.40	210.8	18.70	130.4	21.00	73.9	30.30	-0.6
17.00	201.8	18.80	124.2	22.00	65.5		
17.50	191.1	18.95	116.1	23.00	58.4		
17.80	182.3	19.10	109.4	24.00	51.6		
Titration No. 2							
0.50	283.1	16.00	260.5	36.00	172.1	43.00	73.5
1.00	282.6	18.00	257.0	36.40	161.0	45.00	65.8
1.50	281.9	20.00	253.3	36.70	151.3	47.00	58.6
2.00	281.2	22.00	249.2	37.00	141.1	50.00	47.7
3.00	279.8	24.00	244.7	37.40	128.7	52.00	39.8
4.00	278.4	26.00	239.5	37.60	123.4	54.00	31.5

Table 10 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
5.00	277.0	28.00	233.5	38.00	114.7	56.00	22.5
6.00	275.7	30.00	226.1	38.50	106.1	57.00	17.9
7.00	274.3	32.00	216.4	39.00	99.5	58.00	12.7
8.00	272.8	33.00	210.1	39.50	94.2	59.00	7.4
10.00	270.0	34.00	201.9	40.00	89.9		
12.00	266.9	35.00	190.5	41.00	83.2		
14.00	263.8	35.50	182.5	42.00	78.0		
Titration No. 3							
0.10	235.4	2.80	174.7	3.70	113.7	9.00	65.1
0.50	231.3	2.90	167.4	3.90	107.7	10.00	59.4
1.00	225.1	3.00	159.0	4.20	101.1	11.00	53.5
1.50	217.4	3.10	149.9	4.50	96.5	13.00	40.7
2.00	207.1	3.20	141.5	5.00	90.8	14.20	32.4
2.20	201.6	3.30	133.8	6.00	82.8	15.50	22.6
2.50	190.9	3.40	127.4	7.00	76.4		
2.65	183.7	3.55	119.6	8.00	70.7		
Titration No. 4							
12.00	280.9	24.60	225.6	27.50	143.8	30.00	81.6
14.00	275.3	25.40	216.2	27.65	134.2	31.00	75.2
16.00	269.2	26.00	206.5	27.80	125.7	32.50	67.7
17.00	265.9	26.50	194.7	28.00	116.0	34.00	61.0
18.00	262.4	26.80	184.8	28.20	108.3	36.00	52.3
20.00	254.4	27.00	176.0	28.40	102.5	38.00	43.1
21.00	249.9	27.20	164.4	28.70	96.1	40.00	33.2
22.50	241.8	27.30	157.9	29.10	90.2	42.00	22.5
23.60	234.3	27.40	150.7	29.60	84.9	43.50	13.7

Table 10 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5							
8.00	287.0	21.50	213.7	23.20	134.0	27.00	67.7
10.00	280.9	22.00	203.1	23.30	126.5	28.00	62.3
12.00	274.3	22.30	194.0	23.45	117.0	30.00	51.9
14.00	267.0	22.50	186.0	23.60	109.5	32.00	41.0
15.50	260.8	22.70	175.5	23.80	102.1	34.00	29.2
17.00	253.6	22.80	168.8	24.10	94.2	35.00	23.1
18.50	244.8	22.90	160.9	24.50	87.4		
19.90	233.9	23.00	152.2	25.00	81.7		
20.80	224.3	23.10	142.8	26.00	73.8		
Titration No. 6							
14.00	212.9	17.70	163.3	19.00	114.1	22.00	68.6
15.00	205.7	17.90	156.3	19.30	106.1	23.00	60.1
16.00	195.9	18.10	148.2	19.60	99.4	24.50	48.4
16.50	189.4	18.30	139.7	20.00	92.2	26.00	36.1
17.00	181.0	18.50	131.4	20.50	84.7	27.50	22.7
17.50	169.3	18.80	120.4	21.00	78.6		
Titration No. 7							
4.00	295.9	8.60	227.9	9.20	153.1	9.60	94.6
5.00	287.8	8.80	217.0	9.22	146.1	9.80	88.3
6.00	278.1	8.90	209.3	9.25	136.4	10.00	84.0
6.80	268.6	9.00	198.8	9.28	128.0	10.50	76.0
7.20	262.8	9.05	191.6	9.32	119.4	11.20	67.1
7.60	256.0	9.10	182.0	9.36	112.5	12.00	57.2
8.00	247.6	9.15	169.5	9.42	105.4		
8.40	235.9	9.17	163.4	9.50	99.3		

Table 10 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 8							
0.50	328.6	8.00	261.3	9.10	200.0	9.32	124.5
2.00	321.0	8.20	256.1	9.15	188.9	9.35	117.5
3.50	312.1	8.40	249.8	9.18	179.9	9.40	109.1
5.00	301.1	8.60	242.0	9.21	168.1	9.45	103.9
6.00	291.7	8.80	231.6	9.24	155.3	9.55	97.6
6.50	286.1	8.91	223.6	9.26	146.6	9.70	92.0
7.00	279.6	9.00	214.8	9.28	138.2	10.00	85.2
7.50	271.6	9.05	208.3	9.30	131.0	10.50	77.5
Titration No. 9							
7.50	196.9	9.15	146.8	10.60	94.0	13.50	36.0
8.00	187.9	9.30	139.0	11.00	85.2	14.00	24.6
8.30	180.8	9.50	129.3	11.40	77.2	14.50	12.8
8.60	171.4	9.70	120.9	11.80	69.7	15.00	2.2
8.90	159.3	10.00	110.4	12.40	58.5	15.50	-7.1
9.00	154.5	10.30	101.5	13.00	46.8		

Table 11 EXPERIMENTAL DATA FOR LEAD-GLYCYLGLYCINATE INTERACTION

Titn. No.	Starting Solution A mM	Starting Solution E mM	Starting Solution H mM	Titrating Solution A mM	Titrating Solution B mM	Titrating Solution H mM	Initial Vol. ml	E^{o} mV
1	60.32	6.135	11.27			-40.02	20.01	452.9
2	30.18	3.069	5.637			-40.02	20.01	452.9
3	59.99	12.26	22.53			-40.02	20.01	451.9
4	24.25	12.27	22.54			-40.02	20.01	451.9
5	12.06	12.26	22.53			-40.02	20.01	451.8
6	119.9	12.26	22.53			-40.02	20.01	451.6
7	5.983	12.27	22.54			-40.02	20.01	451.3
8	12.13	6.141	11.28			-40.02	20.01	451.3
9	6.069	3.071	5.641			-20.01	20.01	451.3

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
4.10	170.0	5.70	112.7	6.30	79.2	7.60	49.7
4.60	160.2	5.80	105.8	6.40	75.7	8.00	43.5
4.90	152.4	5.90	99.3	6.50	72.5	8.50	36.1
5.40	132.0	6.00	93.2	6.70	67.2	9.00	29.2
5.20	141.7	6.10	87.9	7.00	60.6	9.50	22.7
5.60	119.6	6.20	83.2	7.30	54.9	10.10	15.8
Titration No. 2							
2.60	138.0	2.95	92.4	3.80	46.3	6.00	-1.8
2.70	127.6	3.00	86.5	4.00	40.5	6.50	-9.0
2.75	121.2	3.10	77.0	4.20	34.9	7.00	-15.2
2.80	114.0	3.20	70.0	4.50	27.2		
2.85	106.4	3.40	60.0	5.00	15.9		
2.90	99.1	3.60	52.5	5.50	6.2		

Table 11 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3							
0.10	235.3	6.00	207.0	11.20	135.3	13.80	68.1
0.50	233.5	8.00	193.2	11.50	119.7	15.00	56.4
1.00	231.0	9.20	181.1	11.80	105.0	16.50	41.9
2.00	226.7	10.00	169.6	12.20	91.4	18.00	27.2
4.00	217.6	10.70	153.9	12.70	81.4	20.00	9.7
Titration No. 4							
1.00	287.2	9.00	210.5	11.70	108.8	16.60	43.7
2.00	277.1	10.00	194.7	11.90	100.4	17.70	29.8
3.00	267.6	10.50	183.2	12.20	93.7	18.50	19.5
4.50	253.9	11.00	165.3	13.00	82.6		
6.00	240.6	11.40	136.4	14.00	72.1		
7.50	226.9	11.60	116.8	15.50	56.6		
Titration No. 5							
4.00	304.0	8.70	238.7	11.20	164.7	11.80	102.8
5.00	291.8	9.50	224.8	11.30	153.5	12.60	88.6
6.00	277.8	10.00	214.5	11.40	138.4	14.00	74.0
7.00	263.5	10.50	201.7	11.50	122.3	16.00	52.3
8.00	248.8	11.00	180.4	11.60	112.4		
Titration No. 6							
0.20	207.5	8.00	172.8	11.40	117.7	13.80	66.1
1.00	205.1	9.00	163.8	11.70	108.0	15.00	54.1
2.00	201.8	10.10	149.4	12.10	96.0	16.20	44.1
4.00	194.5	10.39	144.3	12.50	86.3	17.60	33.5
6.00	185.4	11.00	130.1	13.00	77.1	19.00	24.0
Titration No. 7							
6.00	308.8	10.00	237.1	11.40	141.6	12.20	94.5

Table 11 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No, 7 cont,							
7.50	290.5	10.50	220.1	11.45	129.0	13.00	84.9
8.50	272.5	11.00	196.1	11.50	119.8		
9.00	261.9	11.20	178.1	11.60	110.5		
9.50	250.2	11.30	164.1	11.80	102.5		
Titration No, 8							
0.20	282.7	4.40	210.3	5.70	123.0	6.60	71.4
1.00	269.4	4.80	198.9	5.80	104.1	7.30	58.0
1.70	258.2	5.10	187.1	5.85	98.2	8.00	44.0
2.50	245.5	5.30	175.9	5.90	94.0	8.70	28.2
3.30	232.5	5.50	158.4	6.00	88.3	9.20	16.5
3.90	221.5	5.60	144.0	6.20	80.9	9.50	9.0
Titration No. 9							
5.60	119.7	5.90	79.6	8.00	34.8	10.00	-6.7
5.65	108.0	6.30	66.4	8.80	19.1		
5.75	91.0	7.20	49.3	9.40	6.4		

Table 12 EXPERIMENTAL DATA FOR LEAD-GLYCYLGLYCYLGLYCINATE INTERACTION

Titn.	Starting Solution			Titrating Solution			Initial	E°
No.	A mM	B mM	H mM	A mM	B mM	H mM	Vol. ml	mV
1	20.10	9.807	18.01			-20.01	20.01	451.2
2	10.06	4.907	9.013			-20.01	20.01	450.6
3	5.029	2.454	4.508			-20.01	20.01	450.6
4	2.481	4.904	9.007			-20.01	20.01	451.4
5	5.011	4.901	9.002			-20.01	20.01	450.4

Table 12 cont.

Titn. No.	Starting Sclution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
6	24.99	4.901	9.002			-20.01	20.01	450.2
7	99.51	9.807	18.01			-40.03	20.01	448.8
8	49.75	4.903	9.006			-40.03	20.01	449.1
9	24.88	2.452	4.504			-40.03	20.01	449.7
10	2.481	4.904	9.007			-40.03	20.01	449.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.20	287.2	11.00	226.2	18.20	130.6	21.50	69.3
1.00	281.8	13.00	214.0	18.40	117.4	23.00	60.3
3.00	269.2	15.00	198.5	18.60	105.7	25.00	48.1
5.00	258.0	16.20	185.2	18.90	95.2	27.00	35.0
7.00	247.4	17.00	172.4	19.30	87.6	29.00	21.7
9.00	237.0	17.80	150.6	20.00	80.0		
Titration No. 2							
0.20	274.0	5.20	225.1	8.90	141.0	10.60	65.2
1.00	266.0	6.50	209.9	9.10	117.6	11.70	52.5
2.00	256.2	7.60	192.1	9.30	95.0	13.00	37.3
3.00	246.6	8.20	177.3	9.40	88.9	14.30	21.3
4.00	237.2	8.60	161.3	9.80	77.1	15.30	9.0
Titration No. 3							
0.10	266.2	3.50	199.6	4.55	101.4	5.60	49.9
0.50	259.4	3.90	184.4	4.60	90.2	6.10	39.3
1.00	251.0	4.20	165.4	4.65	82.9	6.70	26.3
1.70	238.9	4.30	155.2	4.75	74.9		
2.30	227.9	4.40	140.2	4.90	68.0		
2.90	215.6	4.50	115.8	5.10	61.7		

Table 12 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 4							
0.20	323.8	6.20	268.9	8.50	202.8	9.15	115.8
1.00	320.0	6.90	254.3	8.70	190.4	9.25	97.9
2.00	313.9	7.50	239.0	8.90	171.7	9.40	88.4
4.00	298.4	7.90	227.2	9.00	156.3		
5.50	280.5	8.20	216.5	9.10	131.2		
Titration No. 5							
0.20	311.6	6.20	239.8	9.10	117.3	11.00	60.9
1.00	306.1	7.20	221.2	9.20	100.5	13.00	36.6
2.50	292.3	8.00	201.8	9.30	91.9		
4.00	273.7	8.80	162.9	9.50	83.7		
5.20	255.7	9.00	138.4	10.00	73.7		
Titration No. 6							
0.10	223.9	6.00	187.2	9.10	110.1	10.50	63.5
1.00	219.7	7.00	175.9	9.30	96.2	11.50	51.9
2.00	214.7	8.00	158.4	9.50	85.7	13.00	37.1
3.50	206.1	8.50	143.3	9.80	75.9	14.50	23.3
5.00	195.8	8.80	129.7	10.00	71.6		
Titration No. 7							
0.10	198.6	7.80	147.2	10.60	68.7	18.20	7.3
1.00	195.6	8.40	134.7	11.50	56.4	19.50	1.3
2.00	191.6	8.80	123.2	12.50	46.3	21.00	-4.7
4.00	182.0	9.10	112.3	14.00	33.8	22.50	-10.1
6.00	168.4	9.50	96.8	15.60	22.2		
7.00	158.5	10.00	81.3	17.00	13.7		
Titration No. 8							
0.20	197.6	4.00	142.3	4.90	82.4	7.40	26.6

Table 12 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 8 cont,							
1.00	191.3	4.30	127.2	5.10	72.3	8.20	16.7
2.00	181.6	4.50	112.6	5.40	62.0	9.20	6.3
3.00	167.9	4.60	104.2	6.00	48.8		
3.60	155.0	4.70	96.1	6.60	38.4		
Titration No. 9							
0.20	193.2	2.05	134.2	2.45	76.3	3.60	27.0
1.00	179.0	2.20	115.4	2.55	66.9	4.00	17.3
1.40	168.3	2.30	98.6	2.70	57.5		
1.70	157.0	2.35	90.2	2.90	48.7		
1.90	146.3	2.40	82.5	3.20	38.4		
Titration No.10							
0.20	323.3	3.50	254.0	4.50	160.4	5.00	77.4
1.00	314.9	3.80	238.3	4.55	135.5	5.50	64.6
2.00	300.3	4.00	225.9	4.60	106.3		
2.70	284.5	4.20	210.3	4.65	95.6		
3.10	271.3	4.35	193.2	4.75	87.6		

Table 13 EXPERIMENTAL DATA FOR LEAD-CHLORIDE INTERACTION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mN	B mM	H mM	Initial Vol. ml	E° mV
1	5.028	4.910	9.018	199.9			20.01	62.50
2		4.910	9.018	199.9			20.01	62.60
3		9.815	18.03	199.9			20.01	63.00
4		6.134	11.27	198.6			20.01	63.00

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	-71.1	1.30	-45.8	2.80	-32.6	6.20	-18.2
0.15	-69.2	1.40	-44.6	3.00	-31.3	6.60	-17.1
0.20	-67.5	1.50	-43.5	3.20	-30.1	7.00	-16.1
0.30	-64.3	1.60	-42.5	3.40	-29.0	7.50	-14.9
0.40	-61.7	1.70	-41.5	3.60	-28.0	8.00	-13.9
0.50	-59.2	1.80	-40.5	3.90	-26.5	8.50	-12.9
0.60	-57.1	1.90	-39.6	4.20	-25.2	9.00	-12.0
0.70	-55.1	2.05	-38.1	4.50	-23.9	9.60	-10.9
0.80	-53.2	2.20	-36.9	4.80	-22.8	10.30	-9.8
0.90	-51.6	2.35	-35.7	5.10	-21.7	11.00	-8.8
1.10	-48.5	2.50	-34.6	5.40	-20.7	11.80	-7.7
1.20	-47.3	2.65	-33.5	5.80	-19.4		
Titration No. 2							
0.40	-81.1	2.50	-39.1	4.10	-28.4	6.80	-18.3
0.50	-75.6	2.60	-38.2	4.30	-27.4	7.10	-17.5
0.70	-67.9	2.70	-37.4	4.40	-26.9	7.40	-16.8
0.90	-62.1	2.80	-36.6	4.60	-26.0	7.70	-16.1
1.10	-57.6	2.90	-35.8	4.70	-25.6	8.00	-15.4
1.30	-53.8	3.00	-35.1	4.90	-24.7	8.30	-14.7
1.50	-50.5	3.10	-34.3	5.10	-23.9	8.60	-14.1
1.70	-47.8	3.20	-33.7	5.30	-23.2	9.00	-13.2

Table 13 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
1.90	-45.2	3.30	-33.0	5.50	-22.4	9.40	-12.5
2.00	-44.0	3.40	-32.3	5.70	-21.7	9.80	-11.8
2.10	-42.9	3.50	-31.7	5.90	-21.0	10.20	-11.1
2.20	-41.9	3.60	-31.1	6.10	-20.4	10.70	-10.3
2.30	-40.9	3.80	-30.0	6.30	-19.8		
2.40	-39.9	4.00	-28.9	6.50	-19.2		
Titration No. 3							
0.10	-114.4	2.10	-43.9	4.00	-29.6	7.70	-16.5
0.20	-98.7	2.20	-42.8	4.20	-28.6	8.10	-15.6
0.50	-77.0	2.30	-41.8	4.40	-27.6	8.50	-14.7
0.70	-69.1	2.40	-40.9	4.60	-26.7	8.90	-13.9
0.90	-63.3	2.55	-39.5	4.80	-25.8	9.30	-13.0
1.10	-58.7	2.70	-38.3	5.05	-24.8	9.80	-12.2
1.30	-54.8	2.85	-37.0	5.30	-23.8	10.30	-11.2
1.50	-51.5	3.00	-35.9	5.60	-22.7	11.10	-10.2
1.60	-50.0	3.15	-34.8	5.90	-21.6	11.70	-9.1
1.70	-48.6	3.30	-33.8	6.20	-20.6	12.50	-8.1
1.80	-47.3	3.45	-32.8	6.50	-19.8		
1.90	-46.1	3.60	-31.9	6.90	-18.6		
2.00	-45.0	3.80	-30.7	7.30	-17.5		
Titration No. 4							
0.30	-87.5	2.25	-41.6	5.00	-24.5	8.40	-14.5
0.45	-78.2	2.40	-40.2	5.25	-23.5	8.90	-13.5
0.60	-71.4	2.60	-38.4	5.50	-22.6	9.40	-12.5
0.75	-66.6	2.80	-36.8	5.75	-21.7	9.90	-11.7
0.90	-62.3	3.00	-35.3	6.00	-20.9	10.30	-11.0
1.05	-59.0	3.20	-33.9	6.25	-20.0	10.70	-10.4

Table 13 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 4 cont.							
1.20	-55.7	3.40	-32.6	6.50	-19.3	11.10	-9.8
1.35	-53.1	3.60	-31.4	6.75	-18.6	11.50	-9.1
1.50	-50.7	3.80	-30.2	7.00	-17.9	11.90	-8.6
1.65	-48.5	4.00	-29.1	7.25	-17.2	12.40	-8.0
1.80	-46.6	4.25	-27.9	7.50	-16.6	12.90	-7.3
1.95	-44.8	4.50	-26.7	7.80	-15.9		
2.10	-43.1	4.75	-25.6	8.10	-15.2		

APPENDIX 3 : CALORIMETERIC DATA FOR LIGAND PROTONATION AND POTENTIOMETRIC DATA AT 10, 25 AND 40°C FOR LEAD(II)-LIGAND INTERACTION

TABLE 1 : CALORIMETRIC DATA FOR GLYCINATE PROTONATION

Titn. no.	Starting Solution AmM	Starting Solution HmM	Titrating Solution AmM	Titrating Solution HmM	Initial Vol. ml
1	25.12	18.79		-150.2	99.57
2	30.18	18.79		-160.2	99.57
3	40.16	18.79		-160.2	99.57
4	20.01	9.399		-160.2	99.57

Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT
Titration No. 1							
1.00	0.0150	8.00	0.0285	20.00	0.0040	27.00	0.0028
4.00	0.0448	12.00	0.0523	22.00	0.0016	30.00	0.0012
6.00	0.0287	16.00	0.0051	24.00	0.0017		

Titration No. 2

1.00	0.0169	7.00	0.0301	17.00	0.0062	29.00	0.0030
3.00	0.0325	9.00	0.0305	21.00	0.0065		
5.00	0.0320	13.00	0.0328	25.00	0.0059		

Titration No. 3

2.00	0.0327	10.00	0.0306	22.00	0.0059	34.00	0.0054
6.00	0.0630	14.00	0.0214	26.00	0.0064		
8.00	0.0309	18.00	0.0071	30.00	0.0053		

Titration No. 4

2.00	0.0314	8.00	0.0039	14.00	0.0028
4.10	0.0344	10.00	0.0034	16.00	0.0025
6.00	0.0182	12.00	0.0031	18.00	0.0020

TABLE 2 : CALORIMETRIC DATA FOR GLYCYLGLYCINATE PROTONATION

Titn. No.	Starting Solution AmM	Starting Solution HmM	Titrating Solution AmM	Titrating Solution HmM	Initial Vol. ml
1	20.10	18.79		-160.2	99.57
2	30.19	18.79		-160.2	99.57
3	25.08	18.79		-160.2	99.57
4	20.07	18.79		-160.2	99.57

Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT
Titration No. 1							
3.00	0.0443	12.00	0.0443	18.00	0,0039	26.00	0.0070
6.00	0.0449	14.00	0.0115	20.00	0.0049		
9.00	0.0441	16.00	0.0055	23.00	0.0058		
Titration No. 2							
3.00	0.0503	12.00	0.0385	21.00	0.0054		
6.00	0.0498	15.00	0.0051	24.00	0.0053		
9.00	0.0492	18.00	0.0055	27.00	0.0052		

Titration No. 3

3.00	0.0505	12.00	0.0334	21.00	0.0054
6.00	0.0498	15.00	0.0057	24.00	0.0059
9.00	0.0484	18.00	0.0053	27.00	0.0057

Titration No. 4

2.00	0.0344	9.00	0.0484	18.00	0.0050
4.00	0.0341	12.00	0.0330	21.00	0.0053
6.00	0.0332	15.00	0.0056	24.00	0.0051

TABLE 3 : CALORIMETRIC DATA FOR GLYCYLGLYCYLGLYCINATE PROTONATION

Titn. No.	Starting Solution AmM	Starting Solution HmM	Titrating Solution AmM	Titrating Solution HmM	Initial Vol. ml
1	25.04	18.79		-160.2	99.57
2	20.12	18.79		-160.2	99.57
3	30.13	18.79		-160.2	99.57
4	25.11	18.79		-160.2	99.57

Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT
Titration No. 1							
3.00	0.0527	12.00	0.0393	21.00	0.0055		
6.00	0.0512	15.00	0.0053	24.00	0.0051		
9.00	0.0504	18.00	0.0054	27.00	0.0059		
Titration No. 2							
3.00	0.0513	9.00	0.0502	15.00	0.0058	21.00	0.0052
6.00	0.0514	12.00	0.0377	18.00	0.0052	24.00	0.0057

Titration No. 3

3.00	0.0503	12.00	0.0320	23.00	0.0061
6.00	0.0496	15.00	0.0054	27.00	0.0068
9.00	0.0488	19.00	0.0072	31.00	0.0060

Titration No. 4

3.00	0.0503	9.00	0.0497	15.00	0.0053	21.00	0.0049
6.00	0.0506	12.00	0.0345	18.00	0.0055	24.00	0.0052

TABLE 4 : CALORIMETRIC DATA FOR GLUTATHIONATE PROTONATION

Titn. No.	Starting Solution AmM	Starting Solution HmM	Titrating Solution AmM	Titrating Solution HmM	Initial Vol. ml
1	20.13	28.19		-250.5	99.57
2	25.12	28.19		-250.5	99.57
3	20.08	18.79		-250.5	99.57
4	25.17	18.79		-250.5	99.57

Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT	Added Vol. ml	ΔT

Titration No. 1

4.00	0.0957	16.00	0.0986	28.00	0.0327		
8.00	0.1045	20.00	0.0852	32.00	0.0292		
12.00	0.1002	24.00	0.0359	36.00	0.0165		

Titration No. 2

4.00	0.0998	16.00	0.0955	28.00	0.0335		
8.00	0.1006	20.00	0.0931	32.00	0.0311		
12.00	0.0978	24.00	0.0461	36.00	0.0282		

Titration No. 3

3.00	0.0798	12.00	0.0761	24.00	0.0337
6.00	0.0784	16.00	0.0864	28.00	0.0297
9.00	0.0767	20.00	0.0381	32.00	0.0177

Titration No. 4

4.00	0.1011	16.00	0.0933	28.00	0.0322
8.00	0.0993	20.00	0.0539	32.00	0.0300
12.00	0.0993	24.00	0.0354	36.00	0.0258

Table 5 POTENTIOMETRIC DATA FOR LEAD-GLYCINATE INTERACTION AT 10 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	10.08	4.913	9.023			-20.04	20.01	419.5
2	10.08	4.912	9.021			-20.03	20.01	420.7
3	5.010	2.455	4.509			-20.03	20.01	420.7

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	277.4	8.85	181.3	9.38	93.5	11.20	38.3
1.00	272.7	8.95	174.3	9.40	89.7	11.60	33.3
2.00	266.9	9.00	170.1	9.42	86.4	12.00	28.3
3.00	260.8	9.05	164.9	9.45	82.5	12.40	22.8
4.00	254.3	9.10	158.6	9.49	78.1	12.80	16.9
5.00	246.9	9.14	152.4	9.54	73.7	13.10	12.1
5.80	240.1	9.18	144.6	9.59	70.1	13.40	6.9
6.50	233.0	9.21	137.4	9.65	66.9	13.60	3.4
7.10	225.7	9.24	129.4	9.71	64.3	13.85	-1.0
7.50	219.8	9.26	123.6	9.80	61.1	14.10	-5.6
7.80	214.6	9.28	118.0	9.90	58.4	14.40	-11.4
8.10	208.4	9.30	112.5	10.10	53.9	14.60	-15.5
8.40	200.3	9.32	107.5	10.30	50.4		
8.60	193.5	9.34	102.5	10.60	46.0		
8.75	186.9	9.36	97.6	10.90	42.1		
Titration No. 2							
1.00	271.9	8.80	190.9	9.47	110.8	10.60	49.3
2.00	266.4	8.95	183.8	9.49	105.4	11.00	43.9
3.00	260.4	9.05	177.8	9.51	100.9	11.40	38.9
3.80	255.4	9.15	169.9	9.53	96.8	11.80	34.1
4.60	249.8	9.20	165.1	9.55	93.0	12.30	27.9

Table 5 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
5.30	244.6	9.25	159.1	9.58	88.1	12.80	21.0
6.00	238.7	9.29	153.1	9.62	83.0	13.20	15.0
6.60	232.8	9.32	147.8	9.66	78.6	13.40	11.8
7.20	225.7	9.35	141.3	9.71	74.5	13.70	6.9
7.70	218.3	9.37	137.0	9.77	70.5	14.00	1.7
8.00	213.0	9.39	131.8	9.85	66.6	14.30	-3.5
8.30	206.5	9.41	126.2	9.95	62.8	14.60	-9.2
8.50	201.3	9.43	121.1	10.10	58.5		
8.65	196.6	9.45	115.6	10.30	54.3		
Titration No. 3							
0.10	268.9	4.35	189.7	4.69	110.3	5.25	41.2
1.00	260.6	4.40	185.4	4.70	104.8	5.40	37.1
1.50	255.4	4.45	180.1	4.71	99.3	5.60	32.3
2.00	249.6	4.50	173.9	4.72	95.0	5.90	25.2
2.50	242.9	4.53	169.1	4.73	89.9	6.20	17.9
2.90	236.6	4.56	163.4	4.74	85.4	6.40	12.7
3.20	231.1	4.58	158.9	4.76	78.4	6.60	6.9
3.50	224.5	4.60	153.7	4.78	73.6	6.80	1.0
3.70	219.3	4.62	147.0	4.80	68.8	7.00	-5.3
3.85	214.6	4.64	137.9	4.83	64.1	7.20	-12.2
3.95	211.1	4.65	132.7	4.87	59.4	7.35	-17.8
4.05	207.1	4.66	127.8	4.92	55.1	7.50	-23.7
4.15	202.5	4.67	122.7	5.00	50.5		
4.25	196.9	4.68	116.4	5.10	46.1		

Table 6 POTENTIOMETRIC DATA FOR LEAD-GLYCINATE INTERACTION AT 25 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	9.863	4.912	9.021			-20.03	20.01	433.6
2	5.063	2.455	4.509			-20.03	20.01	433.7
3	12.31	6.128	10.87			-10.01	20.01	455.5

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	288.7	7.70	223.5	9.09	154.1	9.43	91.7
1.00	283.7	8.00	217.2	9.12	148.5	9.50	85.5
2.00	277.7	8.20	211.9	9.15	142.0	9.60	79.2
3.00	271.3	8.40	205.6	9.18	135.1	9.75	72.6
3.80	265.7	8.60	197.3	9.20	130.5	9.95	66.7
4.60	259.6	8.70	192.4	9.22	125.8	10.20	61.3
5.30	253.7	8.80	186.3	9.25	119.6	10.50	56.2
5.90	248.0	8.90	178.4	9.28	113.4	11.00	49.2
6.50	241.4	8.95	173.6	9.31	108.4	11.50	42.7
7.00	235.0	9.00	167.9	9.35	101.5		
7.40	228.9	9.05	160.8	9.39	95.9		
Titration No. 2							
0.10	277.4	4.30	193.5	4.62	116.0	5.40	42.8
1.00	268.8	4.35	188.3	4.63	111.4	5.60	37.7
1.50	263.3	4.40	182.0	4.64	107.1	5.80	32.8
2.00	257.2	4.45	174.4	4.66	99.1	6.00	27.9
2.50	250.0	4.48	168.5	4.68	92.8	6.20	22.9
2.90	243.3	4.50	164.1	4.70	87.4	6.40	17.7
3.20	237.3	4.52	158.7	4.72	82.9	6.60	12.0
3.40	232.6	4.54	152.3	4.75	76.7	6.80	6.2
3.60	227.2	4.56	144.7	4.78	72.4	6.95	1.5

Table 6 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
3.80	220.8	4.57	139.5	4.82	68.0	7.10	-3.6
3.90	217.0	4.58	135.4	4.90	61.6	7.25	-9.1
4.00	212.7	4.59	130.5	5.00	56.2	7.40	-15.3
4.10	207.5	4.60	125.8	5.10	52.0	7.50	-20.0
4.20	201.4	4.61	120.6	5.25	47.1	7.60	-25.1
Titration No. 3							
8.00	287.0	21.50	213.7	23.20	134.0	27.00	67.7
10.00	280.9	22.00	203.1	23.30	126.5	28.00	62.3
12.00	274.3	22.30	194.0	23.45	117.0	30.00	51.9
14.00	267.0	22.50	186.0	23.60	109.5	32.00	41.0
15.50	260.8	22.70	175.5	23.80	102.1	34.00	29.2
17.00	253.6	22.80	168.8	24.10	94.2	35.00	23.1
18.50	244.8	22.90	160.9	24.50	87.4		
19.90	233.9	23.00	152.2	25.00	81.7		
20.80	224.3	23.10	142.8	26.00	73.8		

Table 7 POTENTIOMETRIC DATA FOR LEAD-GLYCINATE INTERACTION AT 40 C

Titn.	Starting Solution			Titrating Solution			Initial	E°
No.	A mM	B mM	H mM	A mM	B mM	H mM	Vol. ml	mV
1	9.970	4.913	9.023			-20.04	20.01	450.6
2	9.810	4.912	9.021			-20.03	20.01	453.8
3	5.090	2.455	4.509			-20.03	20.01	453.8

Table 7 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	302.1	8.45	213.0	9.27	132.1	10.90	61.8
1.00	296.8	8.60	206.6	9.30	126.9	11.20	57.6
2.00	290.6	8.70	201.4	9.33	122.1	11.60	52.1
2.80	285.2	8.78	196.5	9.36	117.6	12.00	46.8
3.60	279.4	8.85	191.5	9.40	112.4	12.40	41.3
4.30	274.0	8.90	187.6	9.44	107.9	12.80	35.7
5.00	268.0	8.95	182.9	9.49	102.9	13.10	31.3
5.60	262.3	9.00	177.6	9.54	98.7	13.40	26.6
6.10	257.1	9.05	171.2	9.60	94.6	13.70	21.8
6.60	251.1	9.09	165.2	9.70	89.1	14.00	16.7
7.10	244.2	9.12	160.2	9.80	84.8	14.30	11.3
7.50	237.5	9.15	154.8	9.90	81.3	14.60	5.5
7.80	231.6	9.18	149.2	10.00	78.4	14.80	1.3
8.00	226.9	9.21	143.4	10.15	74.7		
8.15	222.9	9.23	139.4	10.35	70.7		
8.30	218.4	9.25	135.7	10.60	66.3		
Titration No. 2							
0.10	304.7	8.60	212.8	9.39	133.7	10.90	66.5
1.00	299.2	8.75	206.1	9.41	130.3	11.30	60.8
2.00	292.9	8.85	200.6	9.44	125.5	11.70	55.5
3.00	286.1	8.95	194.0	9.47	121.2	12.20	48.9
3.80	280.2	9.05	185.6	9.51	115.9	12.70	42.2
4.60	273.9	9.10	180.4	9.55	111.3	13.20	35.1
5.10	269.7	9.14	175.7	9.60	106.2	13.60	29.0
5.60	265.1	9.18	170.5	9.65	102.0	13.90	24.2
6.10	259.9	9.21	165.9	9.72	97.1	14.20	19.
6.60	254.0	9.24	161.1	9.80	92.6	14.50	13.
7.00	248.8	9.27	155.9	9.90	88.2	14.70	9.

Table 7 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
7.40	242.6	9.30	150.6	10.00	84.7	14.90	5.2
7.80	235.3	9.33	144.8	10.15	80.4	15.10	0.3
8.10	228.6	9.35	141.0	10.35	75.8	15.30	-4.9
8.40	220.1	9.37	137.2	10.60	71.2		
Titration No. 3							
0.10	291.8	4.40	196.0	4.68	122.7	5.80	47.2
1.00	282.2	4.45	189.2	4.69	118.9	6.00	42.3
2.00	270.0	4.49	182.8	4.71	112.6	6.20	37.2
2.50	262.7	4.51	179.1	4.73	106.9	6.40	32.0
2.90	255.7	4.53	174.8	4.75	101.9	6.60	26.8
3.20	249.6	4.55	169.9	4.77	97.8	6.80	21.1
3.40	244.9	4.57	163.7	4.80	92.6	7.00	15.1
3.60	239.5	4.58	161.1	4.84	87.0	7.20	8.1
3.80	233.0	4.59	157.7	4.88	82.7	7.35	2.0
3.95	227.2	4.60	153.8	4.93	78.2	7.45	-2.5
4.05	222.6	4.61	150.2	5.00	73.6	7.55	-7.5
4.15	217.2	4.62	146.8	5.10	68.5	7.65	-13.3
4.25	210.4	4.64	138.9	5.20	64.3	7.75	-19.8
4.30	206.3	4.65	134.2	5.40	57.8	7.85	-27.3
4.35	201.6	4.66	130.2	5.60	52.4		

Table 8 POTENTIOMETRIC DATA FOR LEAD-GLYCYLGLYCINATE INTERACTION AT 10 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	10.09	4.912	9.021			-20.04	20.01	419.8
2	10.24	4.912	9.021			-20.03	20.01	418.8
3	5.071	2.455	4.509			-20.03	20.01	419.3

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	254.6	7.50	180.5	9.10	111.0	9.70	56.6
1.00	246.3	7.90	172.7	9.14	104.9	9.90	52.1
2.00	237.4	8.20	165.3	9.17	100.2	10.20	47.1
2.80	230.3	8.40	159.1	9.20	95.1	10.60	41.8
3.60	223.3	8.55	153.4	9.23	90.6	11.00	37.1
4.40	216.3	8.70	146.4	9.27	84.9	11.50	31.4
5.20	208.9	8.80	140.6	9.32	77.5	12.00	25.7
5.90	201.9	8.90	133.4	9.38	71.6	12.50	19.7
6.50	195.0	9.00	123.9	9.45	66.5	13.00	13.3
7.00	188.4	9.05	118.1	9.55	61.6		
Titration No. 2							
0.10	250.3	7.20	183.7	9.10	110.9	9.90	51.3
1.00	242.4	7.60	177.1	9.13	106.5	10.20	46.5
1.50	238.1	7.90	171.1	9.16	102.0	10.50	42.6
2.00	233.9	8.10	166.4	9.19	97.4	10.90	37.7
2.50	229.8	8.30	160.9	9.22	92.4	11.30	33.2
3.00	225.7	8.50	154.0	9.25	87.9	11.80	27.4
3.50	221.5	8.60	149.8	9.30	80.6	12.20	22.6
4.00	217.4	8.70	145.2	9.35	74.2	12.50	18.9
4.60	212.2	8.80	139.5	9.40	69.3	12.90	13.8
5.20	206.7	8.90	132.4	9.45	65.6	13.40	7.2

Table 8 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
5.80	200.7	8.95	128.0	9.50	62.9	13.70	3.2
6.30	195.3	9.00	123.2	9.60	58.7		
6.80	189.0	9.05	117.4	9.75	54.3		
Titration No. 3							
0.10	244.2	3.95	170.8	4.61	98.3	5.05	41.8
0.50	238.3	4.05	166.4	4.62	95.2	5.20	37.4
0.90	232.6	4.15	161.1	4.63	91.6	5.40	32.4
1.30	226.7	4.25	154.7	4.64	87.9	5.70	25.6
1.70	220.5	4.30	150.8	4.66	81.0	5.95	20.2
2.00	215.7	4.35	146.4	4.68	75.0	6.20	14.6
2.30	210.7	4.40	141.0	4.70	69.4	6.40	10.1
2.60	205.4	4.45	134.5	4.72	65.1	6.60	5.4
2.90	199.7	4.48	129.9	4.75	60.4	6.80	0.8
3.20	193.3	4.51	124.6	4.78	56.6	7.00	-3.8
3.45	187.1	4.54	118.4	4.82	52.6	7.20	-8.6
3.65	181.5	4.57	110.9	4.87	49.3		
3.80	176.6	4.59	105.0	4.95	45.4		

Table 9 POTENTIOMETRIC DATA FOR LEAD-GLYCYLGLYCINATE INTERACTION AT 25 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	9.874	4.912	9.021			-20.03	20.01	436.6
2	4.935	2.455	4.509			-20.03	20.01	436.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	266.4	7.00	196.0	9.10	127.8	9.75	68.0
1.00	257.6	7.40	189.7	9.15	122.3	9.90	64.0
1.50	252.7	7.60	186.1	9.20	116.1	10.10	59.9
2.00	247.9	7.80	182.3	9.24	110.8	10.40	55.0
2.50	243.2	8.00	177.9	9.27	106.4	10.80	49.7
3.00	238.6	8.20	172.8	9.30	102.2	11.20	44.9
3.50	233.9	8.40	166.9	9.34	96.7	11.60	40.2
4.00	229.3	8.60	159.7	9.38	91.6	12.10	34.5
4.50	224.5	8.70	155.4	9.42	86.7	12.60	28.7
5.00	219.6	8.80	150.4	9.46	83.1	13.00	23.9
5.50	214.4	8.90	144.4	9.51	79.0	13.40	19.4
6.00	208.9	8.98	138.7	9.57	75.4		
6.50	202.8	9.04	133.6	9.65	71.6		
Titration No. 2							
0.10	256.4	3.80	182.1	4.56	107.4	5.35	41.6
0.50	250.1	3.90	177.9	4.58	101.1	5.55	37.0
0.90	243.6	4.00	173.3	4.60	94.8	5.80	31.5
1.20	238.7	4.10	167.7	4.62	89.2	6.10	25.0
1.50	233.7	4.20	161.1	4.64	83.8	6.40	18.4
1.80	228.6	4.28	154.5	4.66	79.1	6.60	13.9
2.10	223.3	4.33	149.6	4.68	75.1	6.80	9.4
2.40	217.7	4.38	143.8	4.71	70.3	7.00	5.0

Table 9 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
2.70	211.8	4.43	136.5	4.75	65.6	7.20	0.2
3.00	205.3	4.47	129.4	4.80	61.5	7.40	-4.6
3.30	198.0	4.49	125.3	4.90	55.8		
3.50	192.3	4.51	120.6	5.00	51.7		
3.70	185.8	4.54	113.1	5.15	46.9		

Table 10 POTENTIOMETRIC DATA FOR LEAD-GLYCYLGLYCINATE INTERACTION AT 40 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	10.87	4.912	9.021			-20.04	20.01	452.4
2	10.24	4.912	9.021			-20.03	20.01	452.5
3	4.965	2.455	4.509			-20.03	20.01	451.9

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	271.0	7.70	188.4	9.25	116.7	10.50	66.4
1.00	262.4	8.10	179.4	9.30	111.3	10.80	62.2
1.70	255.6	8.30	173.8	9.35	106.1	11.10	58.5
2.40	249.0	8.50	167.0	9.40	101.3	11.50	53.8
3.00	243.5	8.65	160.9	9.45	97.2	11.90	49.3
3.60	237.9	8.75	156.2	9.50	93.5	12.40	43.7
4.20	232.3	8.85	150.8	9.55	90.2	12.90	38.2
4.80	226.4	8.95	144.3	9.65	85.4	13.40	32.6
5.40	220.2	9.05	136.6	9.75	81.7	13.90	27.0

Table 10 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
6.00	213.6	9.10	132.1	9.85	78.7	14.40	21.2
6.60	206.1	9.15	127.3	10.00	75.1	14.90	15.5
7.20	197.4	9.20	122.2	10.20	71.2	15.30	10.7
Titration No. 2							
0.10	275.7	7.40	196.6	9.25	121.6	11.00	59.7
1.00	266.5	7.70	191.0	9.30	116.1	11.40	54.9
1.50	261.6	7.90	186.8	9.35	110.4	11.80	50.4
2.00	256.7	8.10	182.0	9.39	106.2	12.30	44.9
2.50	251.9	8.30	176.5	9.43	102.2	12.80	39.3
3.00	247.1	8.50	169.9	9.47	98.6	13.30	33.7
3.50	242.3	8.65	164.1	9.53	93.8	13.70	29.2
4.00	237.5	8.75	159.6	9.59	90.0	14.20	23.6
4.50	232.5	8.85	154.4	9.70	84.8	14.60	18.9
5.00	227.4	8.95	148.3	9.80	81.1	15.00	14.2
5.50	222.1	9.05	140.8	9.95	76.7	15.50	8.3
6.00	216.4	9.10	136.6	10.10	73.4	16.00	2.2
6.50	210.1	9.15	132.1	10.30	69.6	16.50	-3.7
7.00	203.1	9.20	127.1	10.60	65.0		
Titration No. 3							
0.10	266.1	3.90	185.2	4.67	101.8	6.15	37.9
0.50	259.5	4.05	177.9	4.69	97.4	6.40	32.8
0.80	254.5	4.15	172.2	4.72	91.8	6.65	27.8
1.10	249.4	4.25	165.2	4.75	87.4	6.90	22.7
1.40	244.2	4.32	159.4	4.78	82.8	7.15	17.9
1.70	238.9	4.38	153.4	4.81	79.8	7.40	12.2
2.00	233.5	4.43	147.3	4.86	75.4	7.65	6.8
2.30	227.9	4.47	142.0	4.93	70.7	7.85	2.4

Table 10 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
2.60	221.8	4.51	135.5	5.00	67.1	8.05	-2.0
2.90	215.4	4.54	130.1	5.10	63.0	8.25	-6.4
3.20	208.2	4.57	123.9	5.25	58.2	8.50	-11.9
3.40	202.6	4.59	119.7	5.45	52.9		
3.60	196.5	4.61	115.3	5.65	48.3		
3.75	191.2	4.64	108.1	5.90	43.0		

Table 11 POTENTIOMETRIC DATA FOR LEAD-GLYCYLGLYCYLGLYCINATE INTERACTION AT 10 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	9.400	4.912	9.021			-20.03	20.01	419.6
2	5.096	2.455	4.509			-20.03	20.01	419.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	252.9	7.40	179.6	9.19	108.0	10.05	49.4
1.00	244.3	7.70	174.4	9.23	102.4	10.30	45.5
1.50	239.6	8.00	168.4	9.26	97.8	10.60	41.5
2.00	235.1	8.20	163.6	9.29	92.9	11.00	36.8
2.60	229.6	8.40	157.8	9.32	87.9	11.40	32.2
3.20	224.2	8.55	152.8	9.35	83.2	11.90	26.7
3.80	218.8	8.70	146.8	9.40	76.3	12.30	22.0
4.40	213.4	8.80	141.9	9.43	72.7	12.70	17.3
5.00	207.8	8.90	136.1	9.47	69.0	13.00	13.7
5.60	201.9	9.00	129.0	9.52	65.3	13.30	10.1
6.20	195.5	9.05	124.5	9.60	61.1		
6.70	189.5	9.10	119.4	9.70	57.5		
7.10	184.1	9.15	113.3	9.85	53.4		
Titration No. 2							
0.10	243.0	3.65	177.7	4.54	112.3	4.78	54.0
0.50	236.8	3.80	172.8	4.56	107.3	4.82	50.8
0.80	232.1	3.95	167.0	4.58	101.2	4.87	47.9
1.20	225.8	4.10	159.8	4.60	95.6	4.95	44.2
1.50	221.0	4.20	153.8	4.62	88.8	5.05	40.7
1.80	216.2	4.25	150.3	4.63	85.4	5.20	36.4
2.10	211.2	4.30	146.3	4.64	82.2	5.40	31.6
2.40	205.9	4.35	141.6	4.65	78.5	5.60	27.1

Table 11 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
2.70	200.3	4.40	136.1	4.67	72.7	5.80	22.9
3.00	194.3	4.45	129.3	4.69	67.2	6.00	18.5
3.25	188.6	4.48	124.4	4.71	63.1	6.20	14.2
3.45	183.5	4.51	118.8	4.74	59.0		

Table 12 POTENTIOMETRIC DATA FOR LEAD-GLYCYLGLYCYLGLYCINATE INTERACTION AT 25 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	10.03	4.912	9.021			-20.03	20.01	436.5
2	5.096	2.455	4.509			-20.03	20.01	436.5

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	263.8	6.50	197.9	9.04	126.7	9.70	66.6
0.50	259.6	6.90	192.5	9.09	121.6	9.85	62.7
1.00	254.4	7.30	186.3	9.13	117.3	10.05	58.7
1.50	249.2	7.60	181.0	9.17	112.5	10.30	54.7
2.00	244.2	7.90	174.9	9.21	107.2	10.60	50.6
2.50	239.3	8.10	170.1	9.25	101.9	11.00	45.7
3.00	234.5	8.30	164.5	9.29	96.1	11.50	40.0
3.50	229.7	8.50	157.8	9.33	90.9	12.00	34.5
4.00	224.9	8.60	153.9	9.36	87.3	12.50	28.8
4.50	220.0	8.70	149.5	9.40	83.2	12.80	25.4
5.00	215.0	8.80	144.3	9.45	78.8	13.20	20.8

Table 12 cont.

Added Vol. ml	E mV	Added Vol. ml	E mV	Added Vol. ml	E mV	Added Vol. ml	E mV
Titration No. 1 cont.							
5.50	209.7	8.90	138.1	9.50	75.4	13.60	16.3
6.00	204.1	8.98	132.1	9.60	70.5		
Titration No. 2							
0.10	251.8	3.60	184.1	4.54	116.2	4.95	54.5
0.50	245.2	3.75	179.1	4.56	111.7	5.05	50.6
0.80	240.3	3.90	173.3	4.58	106.8	5.20	46.0
1.10	235.2	4.00	168.7	4.60	101.5	5.40	40.9
1.40	230.1	4.10	163.3	4.62	96.3	5.60	36.4
1.70	225.1	4.18	158.5	4.64	90.6	5.85	31.1
2.00	219.8	4.26	152.6	4.66	85.8	6.10	26.0
2.30	214.3	4.32	147.6	4.68	80.9	6.35	20.7
2.60	208.6	4.37	142.5	4.70	76.6	6.60	15.6
2.85	203.4	4.41	137.8	4.73	71.7	6.85	10.5
3.05	199.0	4.45	132.3	4.76	68.0	7.10	5.5
3.25	194.1	4.49	126.0	4.80	63.6	7.35	0.4
3.45	188.7	4.52	120.3	4.85	59.9	7.60	-4.5

Table 13 POTENTIOMETRIC DATA FOR LEAD-GLYCYLGLYCYLGLYCINATE INTERACTION AT 40 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	10.09	4.912	9.021			-20.03	20.01	453.0
2	5.001	2.455	4.509			-20.03	20.01	453.2

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	274.4	6.90	199.9	9.05	130.1	10.40	65.6
1.00	264.7	7.20	195.1	9.10	125.1	10.80	60.2
1.50	259.3	7.50	189.7	9.15	119.9	11.30	54.2
2.00	254.2	7.70	185.7	9.20	114.4	11.80	48.6
2.50	249.0	7.90	181.3	9.25	108.9	12.30	43.1
3.00	243.9	8.10	176.2	9.30	103.8	12.80	37.7
3.50	238.7	8.30	170.3	9.35	99.1	13.30	32.3
4.00	233.7	8.50	163.2	9.45	91.4	13.80	26.8
4.50	228.6	8.60	159.0	9.53	86.8	14.30	21.4
5.00	223.4	8.70	154.2	9.63	82.4	14.80	16.0
5.50	217.9	8.80	148.8	9.75	78.4		
6.00	212.1	8.90	142.3	9.90	74.6		
6.50	205.6	9.00	134.6	10.10	70.5		
Titration No. 2							
0.10	264.0	3.50	194.5	4.49	126.1	4.95	66.4
0.50	256.9	3.65	189.4	4.52	120.0	5.10	60.8
0.80	251.6	3.80	183.6	4.54	115.4	5.25	56.5
1.10	246.2	3.95	176.8	4.56	111.2	5.45	51.7
1.50	239.0	4.05	171.4	4.58	106.6	5.70	46.3
1.80	233.4	4.15	165.2	4.60	102.1	5.95	41.2
2.10	227.7	4.23	159.1	4.63	96.6	6.20	36.3
2.40	221.6	4.30	152.8	4.67	88.8	6.45	31.4

Table 13 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
2.65	216.3	4.35	147.4	4.70	84.3	6.70	26.8
2.90	210.8	4.40	140.9	4.74	79.8	6.95	21.9
3.10	205.9	4.43	136.4	4.79	75.3	7.15	18.0
3.30	200.5	4.46	131.6	4.85	71.4	7.40	12.9

Table 14 POTENTIOMETRIC DATA FOR LEAD-CYSTEINATE INTERACTION AT 10 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	4.970	1.228	2.256			-20.03	20.01	420.1
2	4.970	1.228	2.256			-20.03	20.01	420.1

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	257.7	2.40	193.5	4.05	148.0	5.20	-8.7
0.50	252.1	2.50	189.7	4.20	142.8	5.35	-19.7
0.90	245.2	2.60	186.3	4.35	136.3	5.50	-28.8
1.10	241.1	2.70	183.2	4.50	127.3	5.70	-39.2
1.30	236.4	2.80	180.4	4.60	117.8	5.90	-48.3
1.50	230.9	2.90	177.7	4.70	100.7	6.20	-60.3
1.65	226.1	3.00	175.1	4.75	83.4	6.50	-71.3
1.80	220.6	3.15	171.3	4.80	59.4	6.80	-81.3
1.90	216.3	3.30	167.7	4.85	40.8	7.10	-91.0
2.00	211.8	3.45	164.0	4.90	28.4	7.40	-100.4
2.10	207.0	3.60	160.0	4.95	19.2	7.70	-109.7
2.20	202.3	3.75	156.3	5.00	11.8	8.00	-119.2
2.30	197.8	3.90	152.5	5.10	0.1	8.30	-128.9
Titration No. 2							
0.10	257.8	3.75	155.9	5.26	-14.4	6.55	-74.3
0.50	252.1	4.10	145.5	5.33	-19.4	6.70	-79.3
1.00	243.2	4.40	132.4	5.40	-24.0	6.85	-84.2
1.50	230.8	4.60	115.2	5.50	-29.9	7.05	-90.6
1.90	215.9	4.70	95.3	5.60	-35.4	7.25	-96.7
2.10	206.5	4.80	53.7	5.70	-40.4	7.45	-102.9
2.30	197.4	4.90	26.0	5.80	-45.3	7.65	-109.1
2.50	189.5	5.00	10.4	5.95	-51.7	7.85	-115.4

Table 14 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
2.75	181.4	5.10	-0.9	6.10	-57.9	8.05	-121.5
3.05	173.3	5.15	-5.4	6.25	-63.6	8.25	-128.0
3.40	164.7	5.20	-9.6	6.40	-69.1	8.45	-134.5

Table 15 POTENTIOMETRIC DATA FOR LEAD-CYSTEINATE INTERACTION AT 25 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	4.788	1.228	2.256			-20.03	20.01	437.0
2	4.788	1.228	2.256			-20.03	20.01	437.2
3	3.898	0.981	1.802			-20.02	20.01	451.3

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	265.4	2.00	219.9	3.25	181.2	4.70	86.4
0.50	259.4	2.10	215.9	3.45	176.3	4.75	60.7
0.80	254.2	2.20	212.0	3.65	171.3	4.80	44.9
1.00	250.3	2.35	206.5	3.85	165.9	4.85	32.9
1.20	245.7	2.50	1.4	4.00	161.3	4.90	24.6
1.40	240.5	2.65	196.8	4.15	155.8	4.95	17.4
1.60	234.4	2.80	192.6	4.30	149.0	5.00	9.6
1.75	229.3	2.95	188.7	4.45	139.4		
1.90	223.7	3.10	184.9	4.60	121.1		

Table 15 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2							
0.10	265.9	3.20	182.5	4.95	19.6	7.10	-76.3
0.50	259.9	3.40	177.6	5.00	13.4	7.50	-87.1
0.80	254.7	3.65	171.4	5.05	8.0	7.90	-97.8
1.10	248.5	3.90	164.6	5.10	3.2	8.20	-106.2
1.30	243.6	4.15	156.3	5.15	-0.8	8.40	-111.8
1.60	234.9	4.40	144.4	5.25	-8.1	8.70	-120.4
1.80	227.9	4.60	125.0	5.45	-20.0	9.00	-129.3
2.00	220.2	4.65	114.3	5.65	-29.5	9.30	-138.4
2.20	212.3	4.70	96.1	5.85	-37.6	9.60	-147.8
2.40	204.9	4.75	69.1	6.05	-44.7	9.90	-157.1
2.60	198.4	4.80	49.4	6.25	-51.5	10.10	-163.3
2.80	192.6	4.85	36.5	6.50	-59.3		
3.00	187.5	4.90	27.1	6.80	-67.9		
Titration No. 3							
0.10	275.4	3.67	138.1	3.91	38.6	5.15	-42.6
1.00	255.2	3.70	130.1	3.94	33.1	5.35	-49.7
1.70	226.3	3.72	122.7	3.97	28.3	5.60	-58.2
2.20	206.2	3.73	117.5	4.00	24.3	5.85	-66.2
2.35	201.3	3.74	111.6	4.04	19.2	6.10	-74.3
2.50	196.6	3.75	104.6	4.08	15.0	6.40	-84.2
2.70	190.6	3.76	97.4	4.13	10.1	6.60	-90.9
2.90	184.7	3.77	90.4	4.19	5.0	6.80	-97.7
3.05	179.7	3.78	83.1	4.26	-0.2	7.00	-104.6
3.20	174.2	3.79	77.2	4.34	-5.7	7.20	-112.0
3.30	170.0	3.80	71.8	4.43	-11.2	7.40	-119.5
3.40	164.8	3.81	67.5	4.52	-16.1	7.60	-127.0
3.50	158.3	3.82	63.3	4.62	-21.0	7.80	-134.5
3.55	154.3	3.84	56.4	4.75	-27.0	8.00	-141.8

Table 15 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
3.60	149.0	3.86	50.3	4.88	-32.3	8.20	-148.6
3.65	141.9	3.88	45.1	5.00	-37.0		

Table 16 POTENTIOMETRIC DATA FOR LEAD-CYSTEINATE INTERACTION AT 40 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	2.460	1.228	2.256			-20.03	20.01	457.8
2	4.854	1.228	2.256			-20.03	20.01	457.8
3	4.854	1.228	2.256			-20.03	20.01	457.9

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	285.8	2.28	220.0	4.20	158.6	4.91	12.0
0.30	282.8	2.36	216.4	4.30	154.3	4.93	6.8
0.60	277.8	2.44	212.9	4.40	149.3	4.95	2.6
0.90	271.9	2.52	209.7	4.50	143.0	4.98	-3.1
1.10	267.3	2.60	206.7	4.60	134.3	5.02	-9.6
1.30	261.9	2.70	203.3	4.65	128.0	5.06	-15.5
1.40	258.7	2.80	200.0	4.70	118.8	5.10	-20.6
1.50	255.4	2.95	195.6	4.75	100.6	5.14	-25.2
1.60	251.9	3.10	191.3	4.80	61.0	5.18	-29.5
1.70	247.9	3.30	185.8	4.82	46.9	5.23	-34.4
1.80	243.9	3.45	181.6	4.84	36.4	5.28	-39.3
1.90	239.1	3.60	177.6	4.85	31.9	5.33	-43.6

Table 16 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
2.00	234.2	3.75	173.3	4.86	27.3	5.40	-49.5
2.10	228.9	3.90	168.9	4.88	20.5	5.50	-57.5
2.20	223.9	4.05	164.1	4.89	17.2		
Titration No. 2							
0.10	278.7	3.00	203.5	4.77	88.2	6.50	-47.8
0.50	272.5	3.15	199.7	4.79	77.1	6.90	-58.9
0.80	267.3	3.30	196.0	4.82	64.0	7.30	-69.6
1.10	260.9	3.50	191.1	4.84	57.8	7.70	-80.2
1.30	255.9	3.65	187.3	4.86	51.7	8.10	-91.0
1.50	250.3	3.80	183.3	4.90	42.6	8.50	-102.1
1.70	244.2	3.95	179.0	4.95	33.4	8.90	-113.7
1.90	237.6	4.10	174.1	5.00	26.3	9.30	-125.6
2.00	233.9	4.20	170.4	5.10	15.3	9.70	-137.7
2.10	230.4	4.30	166.1	5.20	6.8	10.00	-146.7
2.25	225.2	4.40	160.7	5.35	-3.3	10.30	-155.3
2.40	220.1	4.50	153.9	5.50	-11.5	10.60	-163.4
2.55	215.7	4.60	143.8	5.70	-20.6	11.00	-173.2
2.70	211.4	4.70	123.6	5.90	-28.4		
2.85	207.4	4.75	100.5	6.20	-38.6		
Titration No. 3							
0.10	278.3	4.20	169.2	5.45	-10.0	8.60	-105.7
0.40	273.8	4.40	159.3	5.55	-14.9	8.80	-111.4
0.70	268.6	4.50	152.2	5.70	-21.6	9.00	-117.2
1.10	260.4	4.60	141.2	5.85	-27.6	9.30	-126.2
1.50	249.8	4.70	118.7	6.00	-32.9	9.50	-132.3
1.70	243.4	4.75	94.1	6.20	-39.7	9.70	-138.3
1.90	236.5	4.80	68.7	6.40	-45.9	9.90	-144.4

Table 16 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
2.10	229.4	4.85	52.4	6.65	-53.1	10.10	-150.4
2.30	222.6	4.90	41.1	6.90	-59.9	10.30	-156.1
2.50	216.3	4.95	32.5	7.15	-66.6	10.50	-161.7
2.80	207.9	5.00	25.6	7.40	-73.3	10.70	-167.0
3.00	202.8	5.05	19.7	7.60	-78.5	10.90	-171.9
3.30	195.2	5.10	14.7	7.80	-83.7	11.10	-176.6
3.60	187.6	5.15	10.2	8.00	-89.0	11.40	-183.1
3.80	182.3	5.25	2.7	8.20	-94.5		
4.00	176.3	5.35	-4.3	8.40	-100.1		

Table 17 POTENTIOMETRIC DATA FOR LEAD-GLUTATHIONATE INTERACTION AT 10 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	2.480	1.228	2.256			-20.03	20.01	422.1
2	2.480	1.228	2.256			-20.03	20.01	422.6
3	2.480	1.228	2.256			-20.03	20.01	422.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
2.00	236.9	5.85	105.3	6.80	-2.5	7.80	-97.7
2.60	225.0	5.95	96.7	6.85	-9.0	7.95	-105.5
3.20	211.2	6.05	87.1	6.90	-15.7	8.15	-114.6
3.70	197.6	6.15	76.6	7.00	-28.4	8.35	-122.7
4.20	181.4	6.25	65.7	7.10	-40.2	8.60	-131.7
4.80	160.3	6.35	54.3	7.20	-51.2	9.00	-145.7
5.10	148.8	6.45	42.3	7.30	-61.2	9.40	-159.1
5.30	139.9	6.55	30.2	7.40	-70.5	9.70	-169.4
5.50	129.5	6.65	17.3	7.50	-78.5		
5.70	116.8	6.75	4.1	7.65	-88.9		
Titration No. 2							
2.00	234.6	5.25	143.2	6.35	57.1	7.10	-38.4
2.50	225.2	5.40	136.1	6.40	51.0	7.15	-44.3
3.10	212.6	5.55	128.1	6.45	45.1	7.25	-55.0
3.30	207.7	5.70	118.7	6.50	38.9	7.35	-64.8
3.50	202.6	5.75	115.2	6.55	32.7	7.45	-73.1
3.70	197.2	5.80	111.6	6.60	26.2	7.55	-80.1
3.90	191.3	5.85	107.6	6.65	19.8	7.65	-86.5
4.05	186.5	5.90	103.3	6.70	13.0	7.80	-95.6
4.20	181.4	5.95	98.8	6.75	6.4	7.95	-103.2
4.35	176.3	6.00	94.3	6.80	-0.1	8.10	-109.9

Table 17 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
4.50	171.2	6.07	87.5	6.85	-6.5	8.30	-118.3
4.65	165.9	6.13	81.4	6.90	-13.4	8.50	-125.7
4.80	160.7	6.19	75.0	6.95	-19.8	8.80	-136.0
4.95	155.3	6.25	68.4	7.00	-26.2	9.10	-146.3
5.10	149.5	6.30	62.8	7.05	-32.2		
Titration No. 3							
2.20	233.0	6.15	81.1	7.20	-48.7	8.05	-107.8
2.80	220.9	6.25	70.1	7.25	-54.2	8.17	-113.0
3.40	206.5	6.35	58.9	7.30	-59.0	8.30	-117.9
3.90	192.2	6.42	50.7	7.35	-63.8	8.45	-123.5
4.10	185.7	6.50	41.0	7.40	-68.2	8.60	-128.9
4.40	175.3	6.60	28.4	7.45	-72.1	8.75	-134.4
4.70	164.7	6.70	15.5	7.50	-75.9	8.90	-139.3
5.00	153.9	6.80	2.0	7.55	-79.7	9.05	-144.3
5.30	141.2	6.90	-11.4	7.60	-83.1	9.20	-149.3
5.60	125.6	6.98	-22.0	7.67	-87.7	9.35	-154.2
5.80	112.3	7.05	-30.8	7.75	-92.4	9.50	-159.1
5.95	100.4	7.10	-37.4	7.83	-96.9		
6.05	91.1	7.15	-43.2	7.93	-102.0		

Table 18 POTENTIOMETRIC DATA FOR LEAD-GLUTATHIONATE INTERACTION AT 25 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	2.382	1.228	2.256			-20.03	20.01	440.0
2	2.382	1.228	2.256			-20.03	20.01	440.0

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
1.50	254.2	4.85	154.8	6.20	57.6	7.05	-42.5
2.00	245.1	4.95	150.3	6.25	51.9	7.15	-51.8
2.30	238.9	5.10	143.1	6.30	46.2	7.20	-56.0
2.50	234.6	5.20	138.0	6.35	40.0	7.30	-63.7
2.70	230.0	5.30	132.7	6.40	34.0	7.35	-67.4
3.00	222.6	5.40	126.8	6.45	27.8	7.45	-74.1
3.20	217.3	5.50	120.6	6.50	21.4	7.55	-80.1
3.40	211.6	5.60	113.6	6.55	15.0	7.65	-85.5
3.60	205.4	5.68	107.7	6.60	8.7	7.75	-90.7
3.80	198.7	5.75	102.0	6.65	2.5	7.90	-97.7
3.95	193.1	5.82	96.1	6.70	-3.5	8.05	-104.1
4.10	187.2	5.90	88.9	6.75	-9.6	8.20	-110.1
4.25	180.9	5.95	84.2	6.80	-15.7	8.35	-115.7
4.40	174.3	6.00	79.2	6.85	-21.5	8.55	-122.9
4.50	170.0	6.05	74.0	6.90	-27.1	8.75	-129.8
4.60	165.7	6.10	68.7	6.95	-32.5	9.00	-138.2
4.70	161.4	6.15	63.2	7.00	-37.8		
Titration No. 2							
1.30	258.1	5.45	123.4	6.76	-12.2	7.77	-91.7
1.60	252.9	5.60	113.3	6.80	-17.0	7.87	-96.3
1.90	247.4	5.75	101.8	6.85	-22.7	7.98	-101.1
2.20	241.4	5.85	93.1	6.90	-28.4	8.08	-105.2

Table 18 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
2.50	234.9	5.95	83.8	6.95	-33.8	8.20	-110.0
2.80	227.8	6.03	75.7	7.00	-38.8	8.35	-115.6
3.10	220.2	6.11	67.2	7.05	-43.7	8.50	-121.0
3.40	211.7	6.20	57.1	7.10	-48.5	8.65	-126.2
3.70	202.2	6.30	45.6	7.15	-52.8	8.80	-131.2
4.00	191.3	6.40	33.1	7.20	-57.0	8.95	-136.2
4.25	180.8	6.50	20.8	7.26	-61.6	9.10	-141.3
4.45	172.1	6.55	14.4	7.33	-66.7	9.25	-146.2
4.65	163.3	6.60	7.9	7.40	-71.6	9.40	-151.2
4.85	154.6	6.64	2.7	7.48	-76.5		
5.05	145.2	6.68	-2.3	7.57	-81.3		
5.25	135.0	6.72	-7.3	7.67	-86.7		

Table 19 POTENTIOMETRIC DATA FOR LEAD-GLUTATHIONATE INTERACTION AT 40 C

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	2.500	1.228	2.256			-20.03	20.01	460.0
2	2.500	1.228	2.256			-20.03	20.01	460.0

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	286.2	3.80	210.4	5.10	153.6	6.10	89.1
0.50	281.0	4.00	203.4	5.20	148.6	6.17	81.7
1.00	273.8	4.15	197.6	5.30	143.4	8.50	-94.4

Table 19 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
1.40	267.2	4.30	191.4	5.40	138.0	8.65	-99.6
1.80	260.0	4.40	187.0	5.50	132.4	8.80	-104.6
2.20	251.9	4.50	182.4	5.60	126.4	8.95	-109.5
2.60	243.2	4.60	177.8	5.70	120.0	9.10	-114.5
3.00	233.5	4.70	173.1	5.80	113.3	9.25	-119.2
3.20	228.3	4.80	168.3	5.88	107.3	9.40	-124.1
3.40	222.7	4.90	163.5	5.95	101.8	9.55	-128.8
3.60	216.8	5.00	158.5	6.02	96.1	9.75	-135.2
Titration No. 2							
3.50	220.1	5.60	126.7	6.62	34.7	7.71	-60.9
3.70	213.9	5.68	121.6	6.67	28.8	7.81	-66.0
3.85	208.9	5.76	116.1	6.72	22.9	7.91	-70.8
4.00	203.6	5.84	110.4	6.77	17.2	8.02	-75.9
4.15	197.9	5.90	105.9	6.82	11.5	8.14	-81.0
4.30	191.6	5.96	101.1	6.87	5.8	8.26	-85.9
4.40	187.2	6.03	95.3	6.92	0.4	8.40	-91.4
4.50	182.7	6.10	89.0	6.97	-4.9	8.55	-96.8
4.60	178.0	6.15	84.6	7.03	-11.1	8.70	-102.1
4.70	173.4	6.20	79.9	7.09	-16.9	8.85	-107.1
4.80	168.6	6.25	74.9	7.15	-22.6	9.00	-111.9
4.90	163.8	6.30	70.0	7.20	-27.0	9.15	-116.7
5.00	158.9	6.35	64.8	7.25	-31.2	9.30	-121.4
5.10	154.0	6.40	59.4	7.31	-36.0	9.50	-127.6
5.20	148.9	6.45	54.1	7.38	-41.2	9.65	-132.2
5.30	143.8	6.50	48.5	7.45	-46.1		
5.40	138.3	6.54	43.9	7.53	-51.2		
5.50	132.6	6.58	39.3	7.61	-55.4		

APPENDIX 4 : POTENTIOMETRIC DATA FOR BOVINE SERUM ALBUMIN - PROTON, -LEAD(II) AND -COPPER(II) INTERACTIONS

Table 1 EXPERIMENTAL DATA FOR BOVINE SERUM ALBUMIN PROTONATION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	0.058		40.11			-80.07	20.01	413.4
2	0.116		40.11			-80.07	20.01	412.5
3	0.173		40.11			-80.07	20.01	412.6

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
6.50	279.9	9.60	158.4	10.25	-9.9	10.57	-158.4
7.00	272.8	9.70	149.4	10.27	-20.7	10.61	-166.4
7.50	263.6	9.80	137.6	10.29	-31.6	10.66	-175.1
8.00	251.2	9.88	125.5	10.31	-41.9	10.72	-183.6
8.20	244.5	9.93	116.3	10.33	-53.2	10.80	-193.0
8.30	240.6	9.98	105.8	10.35	-64.9	10.88	-200.7
8.40	236.4	10.02	95.8	10.37	-76.4	10.98	-208.7
8.50	231.9	10.05	87.0	10.39	-89.6	11.10	-216.4
8.60	227.0	10.08	76.9	10.41	-103.8	11.30	-226.3
8.70	221.7	10.11	64.7	10.42	-109.6	11.50	-233.9
8.85	213.1	10.13	55.5	10.44	-120.6	11.80	-242.4
9.00	203.9	10.15	45.4	10.46	-129.2	12.20	-251.1
9.15	193.9	10.17	34.3	10.48	-137.0	12.60	-258.0
9.30	182.5	10.19	23.5	10.50	-143.9		
9.40	174.3	10.21	12.2	10.52	-145.7		
9.50	166.2	10.23	0.9	10.54	-151.0		
Titration No. 2							
0.10	317.4	8.80	177.4	10.40	16.8	11.40	-191.2
1.00	312.6	9.00	168.7	10.45	1.4	11.50	-198.0
2.00	307.2	9.20	160.0	10.50	-12.3	11.60	-203.7
3.00	300.4	9.40	150.7	10.55	-26.1	11.75	-211.5

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
4.00	292.1	9.50	145.2	10.60	-39.0	11.90	-217.9
5.00	281.8	9.60	138.7	10.65	-52.4	12.10	-225.4
6.00	267.5	9.70	131.4	10.70	-66.4	12.30	-231.6
6.70	253.1	9.80	123.0	10.75	-82.1	12.60	-239.0
7.00	245.1	9.90	113.4	10.80	-98.9	12.90	-244.9
7.20	239.0	10.00	101.8	10.85	-115.0	13.30	-251.7
7.40	232.2	10.05	95.2	10.90	-128.3	13.80	-258.8
7.60	224.9	10.10	87.8	10.95	-138.9	14.30	-264.5
7.80	217.4	10.15	79.5	11.00	-148.1	14.80	-269.4
8.00	209.9	10.20	69.7	11.05	-156.0	15.80	-277.3
8.20	202.5	10.25	58.6	11.10	-162.7	16.80	-283.0
8.40	194.8	10.30	46.0	11.20	-174.3	18.00	-288.3
8.60	186.4	10.35	31.6	11.30	-183.4		
Titration No. 3							
2.00	300.3	9.20	149.4	10.80	-18.5	11.95	-188.8
3.00	291.8	9.50	136.1	10.85	-27.3	12.00	-190.8
4.00	280.9	9.70	125.2	10.90	-36.8	12.10	-196.3
4.80	269.3	9.85	115.4	11.00	-55.2	12.20	-201.0
5.40	257.7	9.95	108.2	11.10	-75.3	12.40	-209.8
5.80	248.3	10.05	99.8	11.15	-87.1	12.70	-220.6
6.20	237.4	10.15	90.1	11.20	-99.4	13.00	-229.0
6.50	228.6	10.25	78.6	11.25	-111.6	13.40	-237.6
6.80	219.9	10.35	64.7	11.31	-124.3	13.80	-244.2
7.10	211.5	10.40	56.8	11.35	-131.7	14.30	-251.4
7.40	203.6	10.45	48.1	11.40	-139.8	15.00	-259.8
7.70	195.3	10.55	29.0	11.45	-145.9	16.00	-269.3
8.00	186.4	10.60	19.2	11.50	-152.3	17.00	-276.3
8.30	177.1	10.65	9.5	11.60	-162.6	18.50	-284.3

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
8.60	168.1	10.70	-0.4	11.70	-171.6		
8.90	159.3	10.75	-9.4	11.80	-178.9		

Table 2 EXPERIMENTAL DATA FOR BOVINE SERUM ALBUMIN-LEAD INTERACTION

Titn. No.	Starting Solution A mM	E mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	0.115	0.491	41.01			-80.07	20.01	411.5
2	0.115	0.985	41.92			-80.07	20.01	412.6
3	0.116	4.910	49.13			-80.07	20.01	412.8

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	318.3	9.30	164.9	10.83	-13.9	12.00	-206.0
1.00	313.7	9.50	156.7	10.88	-27.2	12.15	-210.9
2.00	307.8	9.70	147.1	10.93	-42.2	12.30	-217.6
3.00	301.2	9.90	134.4	10.98	-59.7	12.50	-225.2
4.00	293.4	10.05	122.5	11.03	-78.9	12.80	-234.1
5.00	283.7	10.15	113.0	11.05	-86.2	13.20	-242.9
6.00	270.7	10.25	101.8	11.07	-93.0	13.70	-251.3
6.80	255.5	10.35	87.7	11.10	-102.0	14.20	-257.9
7.30	242.0	10.40	79.7	11.15	-114.7	15.00	-266.7
7.60	232.2	10.43	74.2	11.20	-126.1	16.00	-274.9
7.90	221.4	10.48	64.3	11.25	-135.6	17.50	-283.6
8.10	213.9	10.53	53.5	11.30	-143.9	19.50	-291.6

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
8.30	206.5	10.58	42.3	11.40	-157.7	22.00	-298.2
8.50	198.9	10.63	31.2	11.50	-168.9	25.00	-303.9
8.70	190.8	10.68	20.4	11.60	-178.4		
8.90	182.2	10.73	9.0	11.70	-186.6		
9.10	173.3	10.78	-2.6	11.85	-197.1		
Titration No. 2							
0.10	320.0	8.80	197.7	11.00	16.0	12.10	-181.6
1.00	315.4	9.00	189.3	11.05	4.5	12.25	-192.4
2.00	309.8	9.30	176.5	11.10	-7.3	12.40	-201.6
3.00	303.6	9.50	168.1	11.15	-19.9	12.60	-211.6
4.00	296.2	9.80	155.7	11.20	-32.5	12.90	-223.3
5.00	287.3	10.00	145.5	11.25	-45.1	13.20	-232.2
6.00	275.3	10.20	132.1	11.30	-57.2	13.60	-240.7
6.50	267.4	10.35	119.6	11.35	-69.3	14.00	-247.5
7.00	257.4	10.45	109.3	11.40	-81.4	14.50	-254.2
7.50	244.3	10.55	97.2	11.45	-93.3	15.50	-265.4
7.80	234.8	10.65	82.8	11.50	-116.0	17.50	-279.4
7.90	231.3	10.70	74.3	11.60	-125.6	18.50	-284.1
8.00	227.5	10.75	65.2	11.70	-141.4	20.00	-289.7
8.10	223.9	10.80	55.6	11.80	-155.0	22.00	-295.3
8.30	216.6	10.85	46.0	11.90	-163.1	25.10	-301.5
8.50	209.2	10.90	36.0	12.00	-173.0		
Titration No. 3							
0.10	328.3	9.40	241.9	11.20	174.3	16.30	-221.0
1.00	324.2	9.60	235.6	11.40	166.2	16.70	-231.3
3.00	314.4	9.80	228.9	15.10	-157.6	17.10	-239.0
4.50	305.8	10.00	221.8	15.20	-166.7	17.60	-246.4

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
5.50	298.9	10.20	214.2	15.30	-174.1	18.30	-255.0
6.50	290.5	10.40	206.4	15.40	-181.2	19.00	-261.6
7.50	279.6	10.60	198.5	15.60	-193.4	20.00	-269.1
8.50	264.1	10.80	190.5	15.80	-203.0	21.50	-277.6
9.00	253.0	11.00	182.4	16.00	-211.0	23.00	-283.6

Table 3 EXPERIMENTAL DATA FOR BOVINE SERUM ALBUMIN-COPPER INTERACTION

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	0.116	0.489	40.53			-80.07	20.01	412.6
2	0.116	0.489	40.53			-80.07	20.01	411.3
3	0.115	0.978	40.95			-80.07	20.01	410.9
4	0.115	2.454	42.21			-80.07	20.01	410.2
5	0.116	0.098	40.20			-80.07	20.01	411.9

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	318.2	8.80	185.1	11.17	-99.0	12.40	-217.1
1.00	313.5	9.00	177.7	11.20	-106.2	12.60	-224.0
2.00	307.7	9.20	169.8	11.25	-116.1	12.90	-232.8
3.00	301.1	9.40	161.6	11.30	-126.1	13.30	-242.2
4.00	293.2	9.60	152.8	11.35	-135.2	13.75	-250.6
5.00	283.4	9.80	142.9	11.40	-143.0	14.30	-258.7
5.80	273.1	10.00	130.7	11.45	-150.2	15.00	-266.3

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1 cont.							
6.40	262.8	10.80	17.6	11.50	-156.5	16.00	-274.9
6.90	251.6	10.85	2.4	11.55	-162.2	17.50	-282.1
7.30	240.0	10.90	-12.6	11.60	-167.6	19.50	-290.4
7.60	230.3	10.95	-28.8	11.70	-177.0	21.50	-296.1
7.90	219.3	11.00	-45.8	11.80	-185.2	24.00	-301.3
8.10	211.7	11.05	-63.1	11.90	-192.0		
8.30	204.1	11.10	-79.0	12.05	-201.1		
8.60	192.6	11.15	-93.4	12.20	-208.7		
Titration No. 2							
0.10	317.2	8.20	206.3	10.96	-55.3	12.05	-203.3
1.00	312.6	8.40	198.6	11.00	-68.5	12.20	-210.4
2.00	306.8	8.60	190.9	11.03	-77.1	12.40	-218.5
3.00	300.2	8.80	183.2	11.07	-88.3	12.60	-225.1
4.00	292.3	9.00	175.5	11.11	-99.0	12.90	-233.7
5.00	282.5	9.20	167.4	11.15	-108.9	13.30	-242.5
5.60	275.0	9.40	158.8	11.20	-119.7	13.70	-249.9
6.20	265.5	9.60	149.6	11.25	-127.9	14.20	-257.4
6.60	257.5	9.80	139.0	11.30	-136.4	14.80	-264.4
7.00	247.7	9.90	132.9	11.35	-144.2	15.80	-271.6
7.20	242.2	10.80	-3.7	11.45	-157.6	16.80	-278.4
7.40	235.9	10.85	-20.7	11.55	-168.3	18.50	-286.7
7.60	228.8	10.87	-25.8	11.65	-177.5	20.50	-293.2
7.80	221.5	10.90	-35.6	11.75	-185.0	23.50	-299.9
8.00	213.9	10.93	-45.9	11.90	-195.3		
Titration No. 3							
0.10	317.8	8.50	204.3	11.95	-119.8	14.00	-236.3
1.00	313.2	8.80	194.0	12.00	-127.1	14.40	-244.1

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
2.00	307.5	9.00	187.2	12.10	-140.6	15.00	-253.9
3.00	301.0	9.30	177.0	12.15	-145.6	15.60	-261.5
4.00	293.4	9.60	166.4	12.25	-156.2	16.40	-269.4
5.10	282.9	9.80	158.9	12.35	-164.7	17.40	-276.7
6.00	271.3	10.10	146.8	12.45	-172.5	19.00	-285.3
6.60	260.9	11.65	-61.2	12.60	-183.1	21.00	-292.5
7.00	252.3	11.70	-69.2	12.75	-192.3	24.00	-300.0
7.50	238.4	11.75	-82.0	12.90	-200.1	27.00	-305.0
7.80	228.8	11.80	-92.5	13.10	-209.0		
8.00	221.9	11.85	-101.6	13.35	-218.3		
8.20	214.8	11.90	-110.8	13.65	-227.7		
Titration No. 4							
0.10	319.6	8.10	232.4	13.45	-152.0	15.50	-234.2
1.00	314.9	8.30	225.9	13.50	-155.3	15.90	-242.3
2.00	309.4	8.50	219.3	13.60	-163.1	16.50	-251.7
3.00	303.2	8.70	212.7	13.70	-170.0	17.20	-260.7
4.00	296.2	8.90	206.3	13.80	-175.6	18.00	-268.3
5.00	287.6	9.20	196.8	14.00	-185.5	19.00	-275.7
5.80	279.0	9.50	187.3	14.20	-194.4	20.00	-281.3
6.60	267.9	9.80	177.8	14.40	-202.0	21.00	-285.8
7.00	260.7	10.10	167.7	14.60	-209.1	23.00	-292.4
7.40	252.1	10.40	157.0	14.90	-219.1	25.00	-297.2
7.80	241.6	13.40	-148.2	15.20	-227.5	27.00	-301.1
Titration No. 5							
0.10	317.3	9.00	166.9	10.60	-28.1	11.55	-194.8
1.00	312.5	9.20	158.0	10.65	-42.2	11.70	-203.7
2.00	306.6	9.40	148.3	10.70	-57.4	11.90	-213.3

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont.							
3.00	299.9	9.60	136.3	10.73	-67.3	12.10	-221.0
4.00	291.8	9.75	124.9	10.76	-78.1	12.40	-230.7
5.00	281.6	9.85	116.0	10.79	-88.1	12.80	-240.6
5.60	273.6	9.95	105.7	10.82	-98.8	13.30	-249.8
6.20	263.5	10.05	93.6	10.85	-108.9	13.80	-257.1
6.70	252.4	10.15	79.1	10.88	-116.7	14.40	-264.3
7.10	241.1	10.20	70.7	10.91	-124.4	15.20	-271.6
7.40	230.9	10.25	61.0	10.95	-133.4	16.20	-278.7
7.60	223.7	10.30	50.3	11.00	-142.3	17.50	-285.5
7.80	216.2	10.35	38.4	11.05	-146.6	19.50	-293.1
8.00	208.4	10.40	25.9	11.10	-153.6	22.00	-299.3
8.30	196.5	10.45	12.9	11.20	-165.8		
8.50	188.2	10.50	-0.6	11.30	-175.7		
8.80	175.6	10.55	-14.5	11.40	-184.1		

APPENDIX 5 : POTENTIOMETRIC RESULTS FOR THE "CHALLENGE" PROJECT

Table 1 EXPERIMENTAL DATA FOR GLYCINATE PROTONATION IN 1M Cl^-

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	10.18		15.04	9.939		-60.14	20.01	416.6
2	19.99		30.07	19.93		-100.2	20.01	414.5
3	49.69		40.11	49.91		-200.1	20.01	414.0
4	99.97		80.18	99.77		-400.3	20.01	413.8

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	292.3	4.75	190.4	5.12	-64.2	7.50	-154.6
0.50	288.4	4.83	178.2	5.14	-67.9	7.80	-160.3
1.00	283.1	4.87	168.6	5.17	-72.7	8.10	-165.9
1.50	277.4	4.90	158.4	5.20	-76.8	8.40	-171.6
2.00	271.0	4.92	148.8	5.25	-82.3	8.70	-177.5
2.30	266.8	4.94	134.3	5.30	-87.1	8.95	-182.7
2.60	262.3	4.96	102.9	5.36	-91.9	9.20	-188.2
2.90	257.4	4.98	43.5	5.43	-96.8	9.45	-193.9
3.20	251.9	4.99	10.2	5.50	-100.9	9.70	-200.0
3.50	245.7	5.00	-8.8	5.60	-105.9	9.90	-205.0
3.70	241.0	5.01	-20.9	5.75	-112.4	10.10	-210.1
3.90	235.7	5.02	-29.3	5.90	-117.8	10.30	-215.1
4.10	229.3	5.03	-35.9	6.05	-122.7	10.50	-220.0
4.25	223.8	5.04	-41.1	6.25	-127.3	10.70	-224.5
4.35	219.5	5.05	-45.2	6.50	-133.8	10.90	-228.6
4.45	214.4	5.06	-49.1	6.75	-139.5	11.20	-234.1
4.55	208.5	5.08	-54.8	7.00	-144.8		
4.65	200.9	5.10	-60.1	7.25	-149.8		
Titration No. 2							
0.10	303.5	5.70	194.4	6.02	-14.9	6.80	-109.9

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
0.50	300.0	5.75	189.3	6.03	-23.7	6.95	-114.7
1.00	295.1	5.80	182.7	6.04	-30.4	7.15	-120.5
1.50	289.6	5.84	176.2	6.05	-36.6	7.35	-125.4
2.00	283.4	5.87	170.0	6.06	-40.9	7.60	-131.2
2.50	276.8	5.90	161.1	6.08	-48.2	7.85	-136.2
2.90	271.1	5.91	158.1	6.10	-53.7	8.15	-141.7
3.30	265.0	5.92	154.4	6.12	-58.6	8.70	-150.8
3.60	260.2	5.93	149.5	6.14	-63.2	9.50	-163.5
3.90	254.8	5.94	143.6	6.17	-69.0	10.10	-172.6
4.20	249.2	5.95	133.2	6.20	-72.4	10.70	-182.2
4.50	242.9	5.96	127.0	6.25	-78.5	11.30	-193.4
4.70	238.0	5.97	114.0	6.30	-82.0	11.90	-206.1
4.90	232.8	5.98	92.1	6.40	-90.1	12.30	-216.0
5.10	226.6	5.99	59.2	6.50	-96.7	12.70	-226.4
5.40	214.1	6.00	22.9	6.60	-101.7	13.10	-235.9
5.60	202.6	6.01	-1.7	6.70	-106.1	13.50	-244.0
Titration No. 3							
0.10	287.0	3.93	160.0	4.40	-85.9	9.00	-175.4
0.34	283.1	3.96	147.7	4.50	-92.3	9.40	-180.9
0.54	279.8	3.98	135.0	4.60	-97.5	9.80	-186.7
0.74	276.5	4.00	108.2	4.70	-102.0	10.10	-191.4
1.00	272.4	4.02	22.2	4.80	-105.9	10.40	-196.5
1.30	267.1	4.04	-14.6	4.95	-110.8	10.70	-202.1
1.60	261.7	4.05	-23.7	5.10	-115.3	10.90	-206.2
1.90	256.1	4.06	-30.4	5.30	-120.4	11.20	-213.1
2.10	252.2	4.07	-35.6	5.50	-124.9	11.40	-218.2
2.30	248.0	4.08	-39.4	5.75	-129.9	11.60	-223.8
2.50	243.3	4.10	-46.3	6.00	-134.4	11.80	-229.8

Table 1 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
2.70	238.5	4.12	-51.8	6.30	-139.4	12.00	-235.9
3.10	226.8	4.14	-56.3	6.60	-143.9	12.20	-241.9
3.40	215.0	4.16	-60.2	7.00	-149.4	12.35	-246.3
3.60	203.7	4.19	-65.1	7.40	-154.7	12.50	-250.3
3.75	191.2	4.23	-70.6	7.80	-159.8	12.70	-255.2
3.83	181.4	4.27	-74.9	8.20	-165.0		
3.89	170.6	4.33	-80.6	8.60	-170.1		
Titration No. 4							
0.10	291.7	3.88	172.0	4.24	-73.3	8.10	-165.3
0.50	283.8	3.92	162.3	4.28	-77.7	8.50	-170.5
0.70	280.0	3.95	151.7	4.34	-83.1	8.90	-175.7
1.00	274.3	3.97	141.1	4.40	-87.6	9.30	-181.1
1.20	270.5	3.99	123.2	4.50	-93.8	9.70	-187.0
1.40	266.6	4.01	73.8	4.60	-99.0	10.10	-193.2
1.60	262.8	4.02	15.0	4.70	-103.4	10.40	-198.6
1.80	258.9	4.03	-6.4	4.85	-109.0	10.70	-204.4
2.00	254.8	4.04	-18.6	5.00	-113.8	11.00	-211.3
2.20	250.6	4.05	-26.6	5.20	-119.3	11.20	-216.7
2.40	246.1	4.06	-32.5	5.40	-124.1	11.40	-222.6
2.60	241.2	4.07	-37.8	5.65	-129.4	11.60	-229.6
2.80	236.0	4.08	-42.0	5.90	-134.1	11.75	-235.3
3.15	225.0	4.10	-48.8	6.20	-139.2	11.90	-241.5
3.40	214.6	4.12	-53.7	6.55	-144.6	12.00	-245.7
3.60	203.2	4.14	-58.5	6.90	-149.6	12.15	-252.2
3.73	192.6	4.17	-63.8	7.30	-155.0		
3.82	181.8	4.20	-68.3	7.70	-160.2		

Table 2 EXPERIMENTAL DATA FOR GLYCINATE PROTONATION IN 1M ClO_4^-

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	9.810		14.23	9.806		-60.14	20.01	415.2
2	5.172		14.23	5.010		-60.14	20.01	415.4
3	20.37		28.44	20.38		-100.2	20.01	416.0
4	49.96		56.83	49.75		-200.1	20.01	416.0
5	100.1		113.6	99.79		-400.3	20.01	415.2

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	288.1	4.64	169.5	4.85	-54.6	6.50	-144.8
0.50	284.1	4.67	161.5	4.87	-61.0	6.70	-147.8
1.00	278.6	4.69	154.0	4.89	-65.8	7.00	-154.0
1.40	273.8	4.70	149.4	4.92	-71.6	7.30	-160.0
1.80	268.5	4.71	144.2	4.95	-76.8	7.60	-165.8
2.20	262.7	4.72	136.2	4.98	-81.0	7.90	-171.6
2.60	256.2	4.73	126.8	5.02	-85.9	8.20	-177.6
3.00	248.9	4.74	113.5	5.07	-90.9	8.50	-183.7
3.40	240.1	4.75	93.5	5.12	-95.2	8.80	-190.2
3.70	232.2	4.76	60.8	5.18	-99.5	9.00	-194.8
3.80	229.1	4.77	24.8	5.25	-104.0	9.20	-199.6
3.90	225.7	4.78	-0.4	5.35	-109.4	9.40	-204.6
4.00	222.1	4.79	-17.2	5.45	-114.1	9.60	-209.7
4.15	215.6	4.80	-28.0	5.55	-118.2	9.80	-214.8
4.25	210.4	4.81	-35.5	5.70	-123.5	10.00	-219.8
4.45	196.2	4.82	-41.7	5.85	-128.2	10.20	-224.6
4.55	185.4	4.83	-46.9	6.05	-133.9		
4.61	175.9	4.84	-51.1	6.25	-138.9		

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2							
0.10	296.6	4.55	196.3	4.81	-50.6	5.51	-136.4
0.50	293.1	4.60	188.4	4.82	-57.7	5.60	-140.7
1.00	288.4	4.64	179.9	4.83	-62.3	5.70	-144.8
1.50	282.9	4.66	174.8	4.84	-67.0	5.85	-150.9
2.00	276.7	4.68	167.9	4.86	-73.5	6.00	-156.8
2.50	269.4	4.70	159.0	4.88	-79.1	6.15	-162.6
3.00	260.6	4.71	153.3	4.90	-83.6	6.30	-168.4
3.30	254.3	4.72	144.3	4.93	-89.6	6.45	-172.7
3.50	249.4	4.73	134.2	4.96	-94.2	6.60	-178.6
3.70	243.8	4.74	116.8	5.00	-99.6	6.75	-184.5
3.90	237.4	4.75	89.8	5.05	-105.4	6.90	-190.7
4.10	229.4	4.76	53.2	5.10	-110.4	7.05	-197.2
4.20	224.5	4.77	12.5	5.15	-114.7	7.20	-203.8
4.30	218.8	4.78	-18.5	5.20	-118.3	7.35	-210.3
4.40	211.7	4.79	-34.2	5.30	-125.1		
4.50	202.3	4.80	-43.4	5.40	-130.9		
Titration No. 3							
0.10	300.7	5.25	205.2	5.84	-60.4	9.10	-163.5
0.50	296.6	5.42	192.4	5.89	-69.8	9.50	-169.4
1.00	290.9	5.52	181.3	5.96	-78.8	9.90	-175.4
1.50	284.7	5.58	171.2	6.05	-87.4	10.30	-181.8
2.00	278.1	5.62	161.8	6.20	-97.7	10.70	-188.5
2.50	271.0	5.66	146.8	6.40	-107.5	11.10	-195.9
2.90	264.9	5.67	141.8	6.55	-113.2	11.40	-202.0
3.20	260.1	5.68	134.4	6.70	-118.1	11.60	-206.4
3.50	254.9	5.69	124.9	6.90	-123.8	11.90	-213.4
3.80	249.4	5.71	79.3	7.10	-128.8	12.10	-218.4
4.10	243.2	5.73	1.9	7.30	-133.3	12.40	-226.0

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3 cont.							
4.30	238.7	5.75	-23.9	7.60	-139.2	12.60	-230.9
4.50	233.7	5.77	-37.1	7.90	-144.6	12.90	-237.8
4.70	228.0	5.78	-41.7	8.30	-151.3		
5.05	215.4	5.80	-49.6	8.70	-157.4		
Titration No. 4							
0.10	307.3	5.15	209.0	5.80	-36.2	9.80	-161.9
0.50	301.5	5.25	203.7	5.83	-47.5	10.20	-166.5
0.90	295.5	5.35	197.1	5.87	-57.2	10.70	-172.4
1.30	289.3	5.45	188.8	5.93	-67.4	11.10	-177.2
1.60	284.5	5.55	177.1	6.02	-77.6	11.60	-183.5
1.90	279.7	5.62	164.5	6.15	-87.9	12.00	-188.9
2.20	274.9	5.64	159.3	6.33	-97.8	12.40	-194.7
2.50	270.0	5.66	152.9	6.43	-102.2	12.80	-201.2
2.80	265.1	5.68	145.2	6.70	-111.6	13.10	-206.7
3.10	260.1	5.69	139.6	6.90	-117.1	13.40	-212.8
3.40	254.8	5.70	134.2	7.10	-122.0	13.65	-218.5
3.70	249.3	5.71	125.2	7.30	-126.2	13.85	-223.4
4.00	243.3	5.72	113.8	7.60	-131.8	14.05	-228.7
4.30	236.6	5.73	91.7	7.90	-136.9	14.25	-234.3
4.50	231.5	5.74	48.3	8.20	-141.4	14.45	-239.9
4.70	226.0	5.75	10.0	8.60	-147.0	14.90	-251.4
4.90	219.4	5.76	-6.1	9.00	-152.2		
5.05	213.5	5.78	-25.7	9.40	-157.2		
Titration No. 5							
0.50	310.9	5.47	183.6	5.95	-75.2	9.70	-162.8
1.00	301.3	5.56	170.4	6.00	-80.1	10.20	-168.8
1.50	290.9	5.60	161.9	6.05	-84.5	10.90	-174.4

Table 2 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont.							
1.80	285.1	5.64	149.4	6.15	-91.4	11.50	-181.8
2.10	279.4	5.66	139.5	6.20	-94.3	11.90	-187.1
2.30	275.7	5.68	123.0	6.30	-99.3	12.30	-192.8
2.50	272.0	5.70	83.5	6.40	-103.7	12.70	-199.2
2.80	266.7	5.72	0.3	6.55	-109.3	13.10	-206.4
3.10	261.1	5.74	-23.0	6.70	-114.0	13.40	-212.7
3.40	255.5	5.75	-29.2	6.90	-119.4	13.70	-220.0
3.60	251.6	5.76	-34.8	7.10	-124.2	13.90	-225.8
3.90	245.5	5.77	-39.6	7.30	-128.3	14.10	-232.2
4.20	238.8	5.78	-43.8	7.60	-133.9	14.30	-239.4
4.40	233.8	5.80	-49.8	7.90	-138.9	14.45	-245.2
4.60	228.3	5.82	-55.0	8.20	-143.4	14.60	-250.9
4.80	222.0	5.84	-59.3	8.55	-148.4		
5.20	204.9	5.87	-64.6	8.90	-152.9		
5.36	194.2	5.90	-69.1	9.30	-158.0		

Table 3 EXPERIMENTAL DATA FOR NICKEL-GLYCINATE INTERACTION IN 1M Cl^-

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E^o mV
1	9.650	4.803	4.555			-20.04	20.01	414.0
2	4.637	4.799	4.551			-20.04	20.01	414.2
3	14.87	4.803	4.555			-20.04	20.01	414.2
4	19.35	4.803	4.555			-20.04	20.01	413.9
5	24.10	4.803	4.555			-20.04	20.01	412.7
6	28.68	9.602	9.105			-20.04	20.01	412.8
7	3.665	1.201	1.138			-20.04	20.01	411.8

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.10	252.2	4.80	154.3	6.35	71.2	11.30	-25.1
0.50	249.2	4.86	147.4	6.60	64.9	11.70	-33.7
1.50	240.7	4.92	140.7	6.90	58.1	12.10	-43.0
2.20	233.5	4.98	134.2	7.20	51.7	12.40	-50.4
2.90	224.5	5.04	128.2	7.60	43.8	12.70	-58.3
3.40	216.1	5.10	122.9	8.00	36.3	13.00	-66.9
3.80	207.3	5.18	116.9	8.40	28.9	13.30	-76.6
4.00	201.7	5.28	110.4	8.90	20.0	13.50	-83.7
4.20	194.8	5.40	103.8	9.30	12.8	13.70	-91.5
4.40	185.7	5.55	96.9	9.70	5.6	13.85	-97.9
4.55	176.7	5.70	91.1	10.10	-1.7	14.00	-105.1
4.65	169.1	5.90	84.1	10.50	-9.3	14.15	-113.1
4.73	161.7	6.10	77.9	10.90	-17.0		
Titration No. 2							
0.10	264.0	4.55	184.5	5.12	104.4	7.00	28.2
0.50	261.0	4.65	174.9	5.20	97.9	7.25	21.3
1.00	256.9	4.70	168.5	5.30	91.0	7.50	14.2

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 2 cont.							
1.80	249.4	4.75	161.1	5.40	85.1	7.75	6.9
2.50	241.2	4.80	152.2	5.55	77.2	8.00	-0.4
3.10	232.5	4.84	144.5	5.70	70.4	8.25	-8.5
3.50	225.0	4.88	136.6	5.90	61.7	8.50	-17.4
3.80	218.0	4.92	129.4	6.10	54.7	8.70	-25.2
4.00	212.2	4.96	123.0	6.30	48.4	8.90	-34.5
4.20	204.8	5.00	117.3	6.50	42.3	9.05	-42.6
4.40	195.0	5.05	111.4	6.75	35.2		
Titration No. 3							
0.10	243.1	5.00	139.8	9.00	35.7	15.55	-79.5
0.50	240.2	5.10	132.4	9.50	28.0	15.90	-86.7
1.00	236.4	5.20	126.1	10.00	20.3	16.30	-95.0
2.00	227.3	5.35	117.9	10.50	12.6	16.70	-103.2
2.60	220.5	5.50	111.1	11.00	4.8	17.10	-111.4
3.20	212.0	5.65	105.2	11.50	-3.2	17.50	-119.6
3.70	202.4	5.85	98.5	12.00	-11.3	17.90	-128.0
4.00	194.7	6.05	92.5	12.50	-19.9	18.20	-134.5
4.20	188.3	6.30	85.8	12.90	-26.9	18.60	-143.5
4.40	180.2	6.55	80.0	13.30	-34.3	18.90	-150.4
4.55	172.5	6.90	72.4	13.70	-41.9	19.20	-157.8
4.70	163.2	7.30	64.4	14.10	-49.8	19.50	-165.3
4.75	159.3	7.70	57.1	14.50	-57.8		
4.80	155.5	8.10	50.2	14.90	-66.0		
4.90	147.7	8.50	43.6	15.20	-72.3		
Titration No. 4							
0.10	236.5	5.20	130.3	10.40	23.8	17.40	-92.9
0.50	233.8	5.35	122.9	11.00	15.2	17.90	-100.8

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 4 cont.							
1.00	230.0	5.50	116.5	11.60	6.3	18.40	-108.3
2.00	221.2	5.70	109.4	12.20	-2.9	18.90	-115.6
2.80	212.0	5.90	103.0	12.70	-10.9	19.50	-124.0
3.30	204.4	6.15	96.2	13.20	-19.2	20.10	-132.1
3.80	194.7	6.40	90.0	13.60	-26.1	20.70	-140.0
4.10	186.6	6.70	83.5	14.00	-33.2	21.30	-147.7
4.30	179.9	7.10	75.6	14.40	-40.5	22.00	-156.7
4.50	171.3	7.50	68.4	14.80	-47.8	22.70	-166.0
4.65	163.9	8.00	59.8	15.20	-55.2	23.30	-174.2
4.80	155.0	8.40	53.4	15.60	-62.4	23.90	-182.7
4.90	148.4	8.90	45.8	16.00	-69.5	24.50	-191.4
5.00	142.0	9.40	38.4	16.50	-78.1		
5.10	135.9	9.90	31.1	16.90	-84.8		
Titration No. 5							
0.10	229.8	5.35	125.1	11.00	21.4	18.40	-95.0
0.50	227.1	5.50	119.2	11.50	14.2	19.00	-102.6
1.00	223.5	5.70	112.5	12.00	6.9	19.60	-109.8
2.00	214.8	5.90	106.5	12.50	-0.7	20.20	-116.6
2.60	208.3	6.15	99.9	13.00	-8.5	20.90	-123.9
3.20	200.0	6.45	93.1	13.50	-16.6	21.70	-131.6
3.70	190.9	6.80	86.0	14.00	-25.0	22.50	-138.8
4.00	183.8	7.20	78.4	14.50	-33.7	23.30	-145.7
4.20	177.9	7.60	71.4	14.90	-40.7	24.20	-152.3
4.40	170.8	8.00	64.9	15.30	-47.6	25.20	-160.4
4.60	162.3	8.50	57.1	15.70	-54.4	26.20	-168.6
4.75	154.8	9.00	49.7	16.20	-62.7	27.20	-177.1
4.90	146.9	9.50	42.4	17.00	-75.1	28.20	-186.2
5.05	139.0	10.00	35.4	17.40	-81.1		

Table 3 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 5 cont.							
5.20	131.7	10.50	28.4	17.90	-88.2		
Titration No. 6							
0.10	247.9	9.80	155.2	16.30	57.6	29.10	-55.0
0.50	246.5	10.00	148.2	17.20	50.1	29.80	-62.4
1.00	244.5	10.20	141.6	18.20	41.8	30.50	-70.5
2.00	240.3	10.40	135.6	19.20	33.7	31.20	-78.2
3.60	232.9	10.65	128.9	20.20	25.8	31.90	-85.9
5.00	225.0	10.95	121.9	20.84	20.7	32.60	-93.9
6.00	218.1	11.30	114.9	21.90	12.2	33.30	-101.9
7.00	209.7	11.70	108.0	22.90	3.9	34.00	-109.9
8.00	198.0	12.20	100.6	23.90	-4.6	34.70	-118.0
8.50	189.9	12.80	92.8	24.90	-13.3	35.40	-125.9
8.80	184.0	13.40	85.6	25.90	-22.4	36.10	-134.6
9.10	177.1	14.00	79.2	26.80	-31.1	36.70	-142.6
9.40	168.1	14.70	72.2	27.60	-39.1	37.30	-150.9
9.60	161.9	15.50	64.9	28.40	-47.4	37.90	-159.7
Titration No. 7							
1.60	46.0	2.55	-15.1	3.53	-78.2	4.38	-135.8
1.70	37.9	2.70	-23.9	3.65	-86.6	4.51	-144.0
1.80	30.9	2.85	-33.0	3.77	-95.1	4.64	-152.1
1.95	20.8	3.00	-42.3	3.89	-103.5	4.77	-160.1
2.10	11.4	3.15	-52.0	4.01	-111.6	4.90	-168.1
2.25	2.4	3.29	-61.4	4.13	-119.8		
2.40	-6.4	3.41	-69.7	4.25	-127.4		

Table 4 EXPERIMENTAL DATA FOR NICKEL-GLYCINATE INTERACTION IN 1M ClO_4^-

Titn. No.	Starting Solution A mM	B mM	H mM	Titrating Solution A mM	B mM	H mM	Initial Vol. ml	E° mV
1	10.34	5.237	4.077			-20.04	20.01	413.8
2	5.249	5.232	4.073			-20.04	20.01	413.7
3	15.78	5.237	4.077			-20.04	20.01	413.5
4	21.11	5.237	4.077			-20.04	20.01	413.5
5	26.07	5.237	4.077			-20.04	20.01	413.6
6	31.40	10.47	8.150			-20.04	20.01	413.3
7	3.984	1.309	1.019			-20.04	20.01	413.3

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 1							
0.30	243.0	4.40	154.0	6.25	71.1	11.50	-23.2
1.00	237.0	4.50	144.7	6.75	60.3	12.10	-35.4
2.00	226.4	4.60	135.8	7.30	49.9	12.50	-44.2
2.70	216.7	4.70	127.8	7.90	39.3	12.90	-53.9
3.30	205.1	4.82	119.7	8.50	29.2	13.50	-70.8
3.70	194.0	5.00	109.9	9.10	19.2	14.10	-92.3
3.95	184.3	5.20	101.2	9.70	9.1	14.40	-106.2
4.15	173.5	5.50	90.7	10.30	-1.2		
4.30	162.7	5.80	82.1	10.90	-11.8		
Titration No. 2							
0.50	253.6	4.36	158.8	5.05	89.8	7.30	21.5
2.00	238.1	4.43	148.0	5.25	80.3	7.70	11.4
3.20	218.4	4.49	138.5	5.50	70.7	8.10	0.5
3.80	200.6	4.55	129.8	5.80	60.7	8.50	-11.4
4.00	191.0	4.63	120.1	6.10	52.0	8.85	-22.9
4.17	179.4	4.73	110.6	6.50	41.5	9.10	-33.3
4.27	170.1	4.85	101.4	6.90	31.4	9.20	-38.0

Table 4 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 3							
0.50	231.5	5.00	118.9	10.20	20.0	16.50	-88.2
2.20	214.8	5.35	106.1	11.20	5.6	17.30	-104.4
3.30	196.8	5.85	92.4	12.20	-9.5	18.10	-121.0
3.90	179.1	6.40	80.0	13.20	-25.8	18.85	-137.4
4.25	162.1	7.20	65.2	14.10	-41.6	19.55	-154.4
4.50	146.2	8.20	49.2	14.90	-56.6	19.90	-163.5
4.70	134.0	9.20	34.4	15.70	-72.2		
Titration No. 4							
1.00	220.6	6.00	96.3	14.10	-26.1	21.60	-140.8
3.00	196.8	6.70	82.5	15.00	-41.2	22.80	-155.7
3.80	178.2	7.50	69.0	15.80	-54.9	24.00	-170.7
4.30	158.1	8.50	54.0	16.60	-68.3	25.20	-186.6
4.50	148.0	9.50	40.1	17.50	-82.9	25.80	-194.7
4.80	133.4	10.70	23.9	18.50	-98.4		
5.10	121.2	11.90	7.3	19.50	-112.4		
5.50	108.6	13.10	-10.3	20.50	-126.5		
Titration No. 5							
0.50	219.6	5.30	118.6	11.80	16.3	19.20	-95.1
1.50	211.1	5.80	105.8	13.00	-0.5	20.40	-110.1
2.90	194.0	6.50	91.5	14.20	-18.9	21.70	-124.4
3.70	177.2	7.30	78.0	15.20	-35.1	23.30	-139.5
4.10	164.6	8.20	64.5	16.00	-48.1	25.20	-155.1
4.50	148.0	9.40	48.0	17.00	-63.8	27.20	-170.9
4.90	131.9	10.60	32.2	18.10	-79.9	29.50	-190.5
Titration No. 6							
0.50	237.1	9.60	139.6	18.80	41.7	32.20	-73.1
2.50	228.2	10.20	126.7	21.00	25.5	33.60	-87.6

Table 4 cont.

Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV	Added Vol.ml	E mV
Titration No. 6 cont.							
4.50	217.0	11.00	113.2	23.00	10.5	35.00	-102.4
6.50	200.6	12.00	100.3	25.00	-5.2	36.40	-117.9
7.70	184.6	13.40	85.8	27.00	-22.2	37.80	-134.3
8.60	165.9	15.00	71.3	28.90	-39.8	39.20	-152.3
9.00	155.3	16.80	56.8	30.60	-56.6		
Titration No. 7							
0.40	208.2	1.20	100.0	2.35	4.2	4.17	-106.8
0.70	193.5	1.26	88.0	2.65	-12.1	4.42	-122.9
0.90	175.9	1.35	75.0	2.95	-28.8	4.67	-138.5
1.00	160.4	1.47	61.9	3.25	-46.3	4.93	-150.8
1.07	141.9	1.62	48.9	3.45	-58.8	5.05	-158.1
1.11	127.9	1.85	33.0	3.69	-74.5		
1.15	113.7	2.10	18.0	3.93	-90.9		

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